Towards tailor-made treatment of early rheumatoid arthritis

Bouwstra, J.K.

2007

document version
Publisher's PDF, also known as Version of record

Towards tailor-made treatment of early rheumatoid arthritis

Omslag: ‘Erwartung’, deel van de Stoclet-fries, Gustav Klimt, 1911, Paleis Stoclet, Wenen

ISBN: 978-90-6464-150-3

De druk van dit proefschrift werd financieel mogelijk gemaakt door Schering-Plough B.V., het Reumafonds en Centocor B.V.

Lay-out en druk: Ponsen & Looijen bv, Wageningen

VRIJE UNIVERSITEIT

Towards tailor-made treatment of early rheumatoid arthritis

ACADEMISCH PROEFSCHRIFT

Ter verkrijging van de graad Doctor aan
de Vrije Universiteit te Amsterdam,
op gezag van de Rector Magnificus
prof. dr. L. Bouter,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der Geneeskunde
op woensdag 4 juli 2007 om 10.45 uur
in het auditorium van de universiteit,
De Boelelaan 1105

door

Jeska Kirsten de Vries-Bouwstra
geboren te Hoogeveen

promotoren:	prof.dr. B.A.C. Dijkmans prof.dr. F.C. Breedveld
copromotoren:	Dr. C.F. Allaart Dr. P.J.S.M. Kerstens

"C'est là, dans cette priorité de l'autre homme sur moi, que,
bien avant mon admiration pour la création,
bien avant ma recherche de la première cause de l'univers,
Dieu me vient à l'idée"

Emmanuel Lévinas (1905-1995)

Contents

Chapter 1

General introduction

Rheumatoid Arthritis

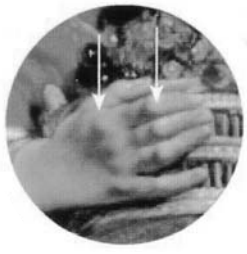
Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic polyarthritis of the small joints of the hands and feet. The inflammation of the synovial membrane of the joint results in hyperplasia of this membrane with destruction of cartilage and bone. Normally, the synovium is an acellular structure consisting of the intimal lining and the synovial sublining. In RA, infiltration of the synovium with T cells, B cells and macrophages results in hyperplasia with proliferation of the synoviocytes of the intimal lining. The resulting mass of tissue is called pannus, which is locally destructive by the release of proinflammatory cytokines and degradative enzymes (1).

RA results in important disabililty. At the short term, disability is caused by joint pain and swelling. At the long term, disability is caused by deformation of joints due to joint destruction. RA is associated with increased risks of coronary artery disease, infection, lymphomas, and reduced life expectancy (2).

History

Examination of skeletal remains of Native Americans living in North America 4000 years B.C. shows clear evidence of RA. However, in antique skeletal remains from Europe no signs of RA can be found. In Europe, first evidence of RA appeared in the 17th century when Sydenham published a case report on the disease. To this day, the highest frequency of RA is found among Native American people, suggesting that RA may have originated in the New World and has somehow arrived in Europe in the wake of the European explorers (1,3).

Figure 1.
'Portret van Jordaens met zijn gezin', (The family of Jordaens), by J. Jordaens, 1620-21, Prado, Madrid.
Findings suggestive of rheumatoid arthritis appear in 17th century European art.



Note swelling of the metacarpophalangeal and proximal interphalangeal joints (arrows).

From the 17th century, in Europe intermittent case reports were published describing features of RA but using other names, like for instance 'asthenic gout' (4). The term 'rheumatoid arthritis' is not used until 1859, when Garrod defined the disease and used this name to distinguish RA from other well-known forms of arthritis: gout and rheumatic fever. In 1957, RA is clearly and definitely defined as a clinical entity distinct from other rheumatic conditions like spondylarthropathies, crystal induced arthritis, and osteoarthritis (5).

Epidemiology

The prevalence and incidence of RA vary from one population to another and from time to time. Among the white populations in Europe and North America, prevalence is approximately 1% and incidence is approximately 0.03% (3). RA is more common in women than in men. In white populations, the peak age of onset is 40 - 60 years, and prevalence increases with age (6).

In The Netherlands, approximately 160.000 people suffer from RA, making it the most common cause of chronic inflammatory arthritis (7). Between 2000 and 2003, observed prevalence in The Netherlands was 6.6 per 1000 men and 11.2 per 1000 women. Yearly, RA is diagnosed in 22.200 patients (0.9 per 1000 men and 1.8 per 1000 women). In 2000, RA was registrated as the primary cause of death in 35 men and 130 women (7,8). Between 2001 and 2004, yearly 3181 patients were hospitalized due to RA (8). Of the total health expenses in 2003 in the Netherlands (€57.5 milliards), €151.3 million was spent on RA (9).

There has been a number of studies showing a decrease of RA incidence in Europe and America, especially in younger women (6,10). However, since RA prevalence increases with age, it is expected that the number of RA patients in The Netherlands will increase with 27% during the coming 15 years, based on demographic changes of the population (7).

Pathogenesis

The exact pathogenesis of RA is still unknown. In general, it is postulated that in a predisposed host an infective or other stimulus binds to receptors on dendritic cells, activating the innate immune system. Dendritic cells migrate to lymph nodes, presenting antigen to T cells. Activated T cells proliferate and migrate into the joint. In the synovial tissue, T cells produce proinflammatory cytokines that stimulate macrophages, fibroblasts, chondrocytes, and osteoclasts, all of which consequently produce a variety of proinflammatory cytokines and degradative enzymes, resulting in cartilage and bone degradation. In addition, activated T cells stimulate formation and maturation of B cells, leading to production of autoantibodies and formation of immunecomplexes and further attraction and activation of macrophages, monocytes, fibroblasts, and granulocytes (1). Maintenance of B cell and T cell activation seems to be mutual dependent, resulting in ongoing perpetuation of the inflammatory response (11,12). Both genetic and environmental factors contribute to development of the inflammatory process as observed in RA (1,10).

Autoantibodies The discovery of autoantibodies, in particular rheumatoid factor (RF), in the blood of patients with RA, contributed to the concept of RA being an autoimmune disease (13). RFs are immunoglobulins (Ig), directed against the Fc-fragment of IgG, which are found in elevated levels in sera of 50% - 80% of RA patients (14). Besides RF, RA patients produce autoantibodies to many different antigens, but only a few are specific for RA, including the autoantibodies to citrulline-containing proteins (14-17). The substrate of anti-cyclic citrullinated protein antibodies (ACPA) is formed by posttranslational de-iminiation of protein-bound arginine. Although the exact role of this modification is not clear, it has been shown that citrullinated proteins are formed during inflammation and are present in the RA synovium (18,19). In addition, the ACPA can be found in serum several years before symptoms of RA occur, indicating a role in RA pathophysiology (20). The sensitivity of ACPA for RA is 54 - 64% and the specificity is about 90 - 97% (21).

Genetic factors Several studies indicated a key role for T cells in the pathogenesis of RA (22,23). Additional evidence for an important role of T cells is the association between the Human Leukocyte Antigen (HLA) complex, also known as the major histocompatibility complex (MHC), and RA development (24). The HLA DR, HLA DQ, and HLA DP loci all encode for HLA class II molecules which are expressed on cells presenting antigens to T cells. The presence of the HLA DRB1 alleles *0101, *0102, *0401, *0404, *0405, *0408, *0410, *1001, or *1402 is associated with susceptibility to but also with severity of RA (25,26). These alleles share a conserved amino acid sequence in their third hypervariable region, the so-called shared epitope (SE) (27). It has been proposed that this region plays a role in the presentation of arthrithogenic antigens to T lymphocytes. As no definite proof of the mechanisms underlying the association between SE and RA is available, other explanations have been proposed (30-32). Interestingly, several studies showed an increased risk for ACPA in RA patients with SE and tobacco exposure, indicating an additional gene-environment interaction (28). In addition, the pattern of inheritance of RA, as shown in family and twin studies, implicates that, apart from HLA class II, other genetic factors play a role in RA development (29-31).

Cytokines Other genetic factors have been suggested to influence the predisposition to RA, including polymorphisms of genes encoding cytokines and degradative enzymes (32,33). Measurement of cytokine levels in RA synovium and synovial fluid showed an imbalanced cytokine profile, with relatively low levels of T cell cytokines, like interleukin 2 (IL2) and interferon γ (IFN-γ), and high levels of cytokines and enzymes produced by macrophages and fibroblasts, like tumor necrosis factor-α (TNF-α) and interleukin 1 (IL1) (34,35). The clinical effectiveness of the cytokine inhibitors anti-TNF-α and interleukin 1 receptor antagonist (IL1Ra), underlined the importance of cytokine networks in the perpetuation of the inflammatory process in RA (36-39).

Clinical characteristics

The main clinical feature of RA is a symmetric polyarthritis involving the small joints of the hands and feet, particularly the metacarpophalangeal, the proximal interphalangeal, the metatarsophalangeal, and the wrist joints. Larger joints and the ligament adjacent to the first and second vertebra of the neck may also be inflamed. On clinical examination, there is swelling and tenderness of palpitation with motion impairment of the affected joints. Ongoing inflammation of the joints eventually results in destruction of cartilage and bone and thus deformation of joints. In daily life, functional ability of RA patients can be severely limited due to pain and impaired function of the affected joints. In addition to joint complaints, patients often experience general symptoms such as morning stiffness, fatigue, fever, and weight loss. Since RA is a systemic disease, many other organs can be affected including the heart, the lungs, the blood vessels, the nerves, and the kidneys, resulting in a variety of symptoms and reduced life expectancy.

In individual patients, the course of RA is variable with periods of remission and exacerbation. Outcome also varies between patients from a remitting disease without joint damage to a severe disease resulting in important disability and death (3).

Diagnosis

Already at presentation there is a great variety in signs and symptoms of patients with RA. In addition, there is overlap of signs and symptoms between patients with RA and patients with self-limiting forms of arthritis or other systemic inflammatory diseases like crystal-induced arthritis.

To distinguish RA from other inflammatory diseases, RA classification criteria have been developed by the American College for Rheumatology (ACR) (40). The 1987 revised ACR criteria include: 1. morning stiffness for at least 1 hour, 2. arthritis of at least 3 joint areas simultaneously, 3. arthritis of hand joints, 4. symmetric arthritis, 5. rheumatoid nodules, 6. presence of rheumatoid factor in serum, and 7. erosive changes on hand and wrist radiographs. According to these criteria, a patient can be classified as having RA when at least four out of seven criteria are present, and of these, the criteria 1, 2, 3, and 4 have to have been present for at least 6 weeks.

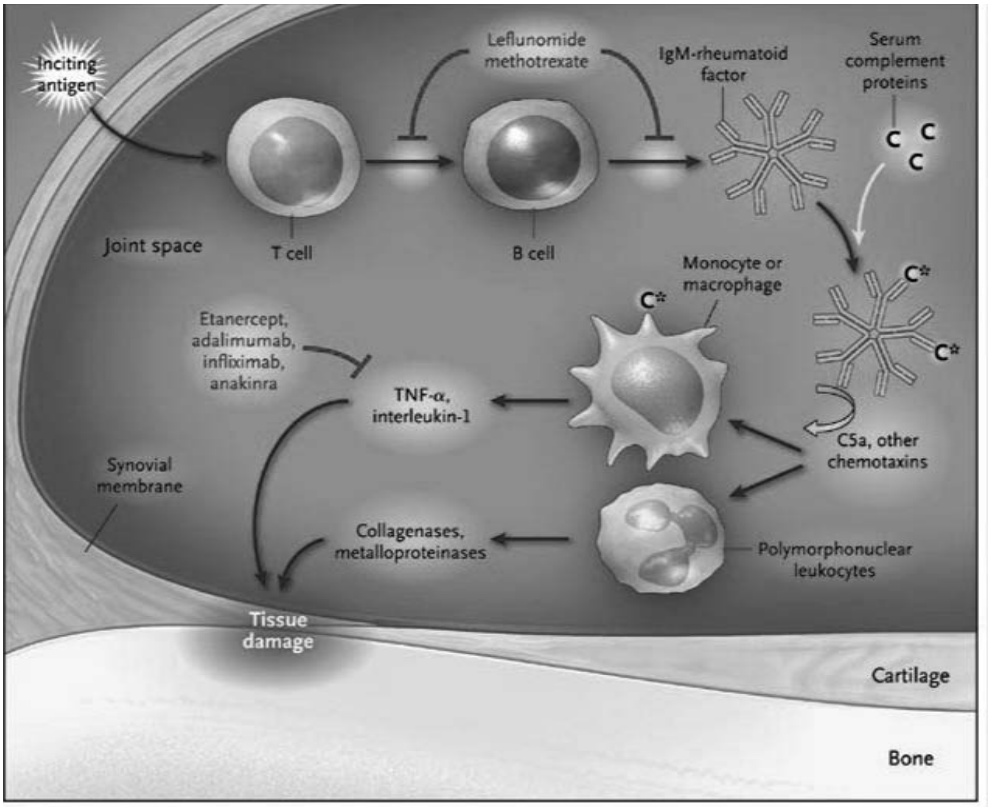
The ACR classification criteria were not designed for diagnosing RA. It has been shown that in patients with symptom duration of less then one year the criteria are relatively insensitive (41,42). Some patients experience fluctuating arthralgia for several months without evidence of joint damage or autoantibodies, but eventually develop RA. Other patients present directly with erosive joint disease, indicating that the pathophysiological process has been ongoing for some time already.

To improve diagnostic abilities in early RA, characteristics of early disease and possible predictors of RA in patients with undifferentiated arthritis (UA) at presentation are intensively studied. Follow-up of patients with UA at presentation showed that 6 - 55% of these patients will develop RA according to the ACR criteria (43). Multivariate

analyses showed that presence of ACPA has the highest sensitivity and specificity for development of RA (14,44,45). However, not all RA patients express ACPA (46). To date, no set of characteristics has been identified that can accurately predict which patients with recent-onset arthritis will develop RA. Therefore, the ACR classification criteria are commonly used as inclusion criteria for clinical trials evaluating treatment efficacy in (early) RA to enhance comparability of study results (47).

Treatment of patients with RA

To date, no curative treatment for patients with RA is known. Nevertheless, significant improvement of outcomes have been achieved due to changes in the therapeutic approach of RA during the last decades.



Olsen N and Stein C. N Engl J Med 2004;350:2167-2179

Figure 2. Inflammation in the rheumatoid joint. The identity of the inciting antigen is not known, but it most likely drives lymphocyte proliferation, which contributes to the production of the rheumatoid factor autoantibody. The fixation of complement amplifies the destructive cascade, attracting additional inflammatory cells and resulting in the production of cytokines and enzymes. These, in turn, mediate tissue damage, including cartilage loss and bone erosion. Likely sites of action of the major drugs described in this chapter are shown. C denotes serum complement protein, and C* activated serum complement protein.

The traditional approach of RA emphasized the stepped use of one medication at a time. When first-line agents, nonsteroidal antiinflammatory drugs (NSAIDs), failed after 6 - 12 months, so-called 'second-line', or 'disease modifying' antirheumatic drugs (DMARDs), were considered. The use of these agents, including antimalarials, sulphasalazine, methotrexate, prednisone, gold salts, cyclosporine, leflunomide and azathioprine, was largely empiric and still little is known about the exact mode of action. Most of these drugs, with the exception of prednisone, have a delayed onset of action (several weeks to months). It has been shown that these agents, in contrast to NSAIDs, are able to slow the rate of joint damage progression (48-54).

Several study results have made this stepwise approach obsolete. As mentioned before, joint damage often occurs early in the course of RA, during the first 2 years (55). It has been shown that it is possible to achieve reduction of joint damage by starting immediately with DMARDs (56,57). Besides, the toxicity profile of NSAIDs and DMARDs has been shown to be similar (58), saving no benefits for the stepwise approach. To improve outcome even further, additional effectiveness of combinations of DMARDs has been studied (59-61). The underlying idea of combination therapy is that by combining several moderately effective drugs (with different mechanisms of action) one can hope to achieve more rapid and greater benefit than that obtained with the same agents used sequentially, based on synergy (1 + 1 > 2), or at least addition (1 + 1 = 2) (62). Several trials showed clear benefit of combinations of DMARDs as compared to DMARD monotherapy without an increase in toxicity (63,64). The COBRA study compared a combination of methotrexate, sulphasalazine, and an initial high dose of corticosteroids, with sulphasalazine alone in patients with early, DMARD-naive RA (65). The combination offered additional disease control over and above that of sulphasalazine alone and, again, with no increase in toxicity. Long-term follow-up patients participating in the COBRA study demonstrated that the benefit of initial combination therapy resulted in prolonged retardation of joint damage progression, regardless of the choice of treatment following the initial therapy (66).

Among the new therapeutic approaches of RA, the introduction of the biologic agents has set complete new standards for treatment of RA. The term *biologics* refers to a group of therapeutic agents that specifically target a particular cell or cytokine important in the inflammatory process in RA (67). Whereas traditional DMARDs generally slow joint damage progression, the prevention of joint damage has become a revolutionary possibility, particularly with the biologics inhibiting TNF-α. Currently, infliximab, etanercept, and adalimumab, all inhibiting TNF-α, are registrated for treatment of RA. These TNF-α inhibitors suppress disease activity directly and powerfully and thus lower the disease burden significantly from the moment that treatment is started (68-70). Patients treated with TNF-α inhibitors show relatively few adverse events, which together with the high clinical effectiveness, is directly favorable for treatment compliance.

Therapeutic strategies

Several agents are effective in treating RA, but treatment response varies between individual patients (3). At present, it is not possible to predict the individual patient's

response to a specific DMARD, making the treatment of patients with RA mainly a case of trial and error. In addition, after achieving an initial good response to treatment with a certain DMARD, the disease activity may relapse after some time, causing a switch in therapy. The sequence of DMARDs in monotherapy and the frequency of use of combinations as well as their compositions differ between countries and between individual rheumatologists (71-73). The reports on the success rate and drug survival of DMARDs vary depending on the definition of 'success' (74,75).

As mentioned above, several trials showed superior efficacy of treatment of early stage RA with a combination of DMARDs or with a TNF-α inhibitor as compared to DMARD monotherapy (70,76-79). Despite these data, there is no consensus whether combination therapy should be started in all patients newly diagnosed with RA. There is a tendency to reserve combination therapy for the patients with clinical characteristics associated with a poor prognosis (80,81). Therefore, in daily clinical practice, usually treatment is started with one DMARD, with methotrexate being the anchor drug (52,62). NSAIDs and/ or a low dose prednisone are sometimes added to offer rapid relief of pain and stiffness.

Measurement of disease course and outcome

In daily clinical practice, the patient and the rheumatologist jointly assess the effectiveness of the prescribed treatment, taking into account the reduction of symptoms, the obtained level of functional ability, and any possible side effects. The individual expectations of both the rheumatologist and the patient influence these assessments and the subsequent decisions to change the medication or not. Therefore, more objective and reproducible monitoring of disease activity and treatment response

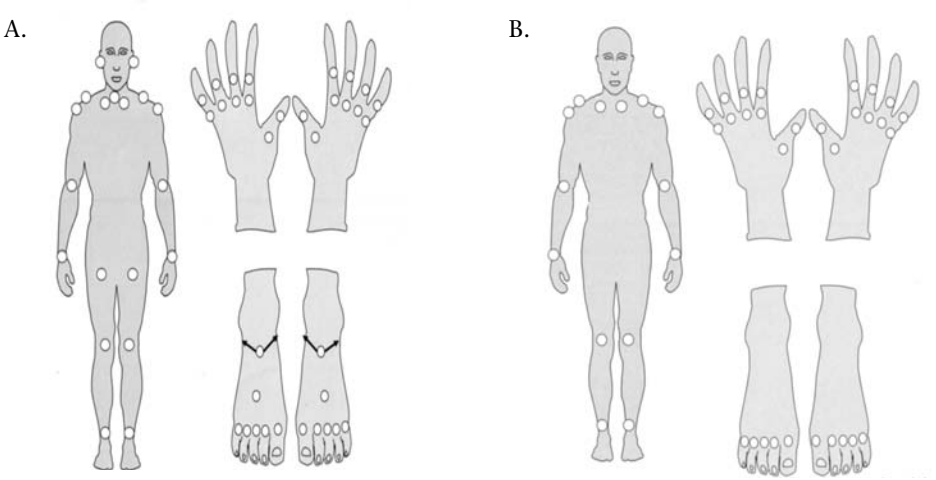


Figure 3. A. The Ritchie Articular Index (RAI): 53 joints are assessed for tenderness on a scale 0 – 3 (with 0 = no tenderness, 3 = winced and withdrew). B. The 44 Swollen Joint Count (SJC): 44 joints are assessed for swelling on a 0 – 1 scale.

is required for evaluation of treatment efficacy in clinical trials. Previous international discussions among rheumatologists have resulted in the description of a core set of measurements to be included in trials concerning RA: number of swollen and number of tender joints, patient's assessment of pain, patient's and assessor's global assessment of disease activity, acute phase response, functional assessment, and radiographic assessment (82,83). Response criteria defined by the American College of Rheumatology (ACR) based on these core set variables, define improvement at different levels: 20% improvement is achieved if a patient improves for 20% or more as compared to baseline in 5 out of 7 core set variables including at least 20% improvement in tender and swollen joint count (ACR20) (84).

To measure disease activity, radiographic joint damage and functional ability in a standardized way, several techniques, and questionnaires have been developed.

Disease activity The disease activity score (DAS) is a composite outcome measure to assess present disease activity, combining the number of tender joints (according to the Ritchie Articular Index [RAI], Figure 3), the number of swollen joints (according to the Swollen Joint Count [SJC], Figure 3), the Erythrocyte Sedimentation Rate (ESR), and the score for general well being by the patient on a visual analog scale (VAS; a 0-100 mm scale, where 0 = best and 100 = worst). All variables are combined in the following equation:

$$DAS = 0.54 \sqrt{RAI} + 0.065\ (SJC) + 0.33 \ln ESR + 0.0072\ VAS\ general\ well\ being.$$

The DAS was developed based upon treatment decisions of rheumatologists in daily clinical practice and has been validated in clinical trials (85,86). As the DAS is a continuous variable, it can be used to determine mean disease activity over a period of time. To assess treatment response based on DAS, response criteria have been defined using attained level and change in DAS (87) (Table 1). In addition, a cut-off level for remission of disease activity has been defined: DAS < 1.6 (88). Also, a modified DAS that requires more limited joint examination, the DAS_{28} - including 28 joints for examination, excluding the ankle and feet joints -, has been developed and validated in clinical practice (89).

Recent clinical trials showed that intensive monitoring of disease activity by using regular DAS assessments and adjusting treatment accordingly results in more intensive treatment and remarkably better outcome for RA patients (90).

Table 1. Disease Activity Score (DAS) improvement criteria

DAS at endpoint	DAS_{28} at endpoint	Improvement in DAS or DAS_{28} from baseline		
		> 1.2	> 0.6 and ≤ 1.2	≤ 0.6
≤ 2.4	≤ 3.2	good		
> 2.4 and ≤ 3.7	> 3.2 and ≤ 5.1		moderate	
> 3.7	> 5.1			none

Functional ability In RA patients, functional ability is assessed using validated questionnaires. The instrument that is currently most used, is the Health Assessment Questionnaire (HAQ) (91,92). The HAQ is a self-administered questionnaire that evaluates the level of difficulty the patient is experiencing with activities of daily living (ADL), and the degree of assistance required by the patient. It has been shown that the HAQ score is valid, sensitive to change, and a strong predictor of future disability in patients with RA (93). The HAQ grades functional ability on a continuous scale from 0 – 3 (0 = best, 3 = worst). A difference of at least 0.20 is regarded to be of clinical significance (94). Functional capacity is strongly influenced by disease activity throughout the course of RA (93). In early RA, functional capacity is not influenced by joint destruction, while in longstanding disease, destruction contributes increasingly to loss of functional ability (93,95,96).

Radiographic joint damage The amount of joint damage is assessed on radiographs, showing periarticular osteoporosis, joint space narrowing, and erosions, as a result of the breakdown of cartilage and bone. Substantial radiographic damage is present in the majority of RA patients and is associated with worse outcome (97,98). Whether progressive joint damage depends on the presence of joint inflammation, or is a process (partly) independent from inflammation, has been a matter of debate for a long period (99,100). Recently, this debate was raised again as data from several clinical trials suggested that treatment with TNF-α inhibitors may prevent the progression of joint damage despite the lack of clinical response (ACR20) (101,102). On the other hand, clinically relevant progression of joint damage can still occur in RA patients with prolonged remission (103).

As radiographic evidence of joint destruction is a key measure of disease outcome, it is crucial that progression of damage, both in early and advanced stages, can be measured reliably and in a reproducible manner. Different methods have been developed and validated in the past (104). Currently, the Sharp-van der Heijde method is used most widely. According to this method, joint space narrowing and erosions are assessed on radiographs of the hands, wrists, and feet (105,106). The maximum total score is 448, with a maximum of 280 for erosions (hands 160, feet 120) and a maximum of 168 for narrowing (hands 120, feet 48). In order to assess progression of joint damage, radiographs of different time points can be scored in single and in random order (single radiographs are randomly ordered with regard to patient and sequence), paired and in random order (two radiographs of the same patient in random order), or paired and ordered (two radiographs of the same patient are presented in chronological order). Paired radiographs have the advantage that the position and the anatomy can be compared. Scoring chronologically is more sensitive to change, but can increase observed progression rates as observers expect scores to increase over time. However, in a trial this effect will apply to all treatment groups equally and so will not bias the treatment comparison. Nevertheless, most groups prefer scoring with order unknown (107,108).

Table 2. Overview of studies evaluating prognostic factors in early RA; Radiographic outcome and functional outcome

Study Author, yr (ref)	No. of patients	Follow-up (yr)	Outcome parameter	Proportion with outcome	Prognostic factors	Characteristics of predictive model
Radiographic outcome						
Combe, 2001 (111)	191	3	Progression SHS > median progression	41%	Erosive disease, IgM RF positivity, ESR ≥ 33, DRB1*04 carrier	Sens 71%; spec 74%
Lindqvist, 2003 (112)	183	10	Larsen progression: 0-5 yr ≥ 11 5-10 yr ≥ 11	 76% 50%	For both outcomes: Mean ESR 0-3 months, RF positivity; for Larsen 0-5 yr: SE carrier	Accuracy 0-5 yr/ 5-10 yr: 93%/97%
Forslind, 2004 (113)	379	2	Larsen score 2 year > 10 Larsen progression > 8	N.A. N.A.	For both outcomes: Larsen at baseline, ACPA positivity, ESR	Accuracy/ PPV/ sens/ spec: 2-yr: 78%/ 79%/ 79%/ 77%; Progression: 75%/ 77%/ 75%/ 75%
Dixey, 2004 (114)	866	3	Larsen Erosive	70%	RFpositivity , ESR	Accuracy/ sens/ spec/ PPV: Erosive:67%/52%/78%/68%
			Larsen erosion score at 3 yr	-	SJC, Larsen, nodules	Erosion score: 82%/96%/42%/77%
Lindqvist, 2005 (115)	183	10	Larsen score 5 yr	-	ESR, COMP, IgA RF, ACPA, anti IL1α	R^2 44%
			Larsen score 10 yr	-	CRP, ACPA	R^2 32%
Functional ability						
Lindqvist, 2002 (116)	183	10	HAQ ≤ 1	47%	Mean HAQ 0-3 months	PPV 70%
Bansback, 2006 (117)	985	5	HAQ ≥ 1.5	298 (30%)	HAQ, Functional class at baseline and 1 yr, DAS_{28} 1yr, Hb, Larsen score, Carstair index	R^2 39%
Combe, 2003, (118)	156	5	HAQ	-	HAQ, ESR, CRP, RAI, erosive	PPV 46%, NPV 93%

Table 2. Overview of studies evaluating prognostic factors in early RA; Remission

Remission						
Gossec, 2004, (119)	191	5	DAS < 1.6 at 3yr	25%	DAS, SHS,	N.A.
			DAS < 1.6 3 & 5yr	16%	DAS, SHS, CRP	
Forslind, 2007 (120)		5	DAS_{28} ≤ 2.6			N.A.
			At one point	35-39%	Gender, RF, DAS_{28}, disease duration	
			At ≥ 2 points	20-26%	Gender, RF, DAS_{28}, disease duration and HAQ	
Eberhardt, 1998 (121)	183	5	ARA remission at any point (excl. fatigue)	37 (20%)	RF, homozygous SE	26% correct

Explanation of abbreviations: N.A.: not analyzed; HAQ: health assessment questionnaire; SHS: Sharp-van der Heijde Score; ESR: erythrocyte sedimentation rate; CRP: C-reactive proteine; RF: rheumatoid factor; SE: shared epitope; ACPA: anti cyclic citrullinated antibodies; SJC: swollen joint count; RAI: Ritchie Articular Index; anti IL1α: anti Interleukin 1α; Hb: hemoglobine; sens: sensitivity; spec: specificity; PPV: positive predictive value; NPV: negative predictive value.

Prognostic markers

Given the heterogeneity of RA, many efforts have been put into the identification of baseline characteristics, serologic and genetic markers predicting a mild or a more severe disease course. The current developments in the treatment of RA increase the interest in identification of different subsets of RA patients early in the disease course. If we could accurately identify those patients that do not develop joint damage and/ or those that can achieve remission of disease activity only given early, aggressive suppression of disease activity, tailor-made treatment of the individual would be possible. An ideal prognostic marker is reliable and reproducible, independent of disease stage, simple to apply in clinical practice, and accurate. The latter indicates that presence or absence of that marker results in a high likelihood of severe outcome, and thus can serve as a starting point for treatment decisions (109).

Joint damage, functional ability, and disease activity are all used as outcome parameters (95,109,110). An overview of prognostic factors in early RA for progression of joint damage, functional disability, and clinical remission is given in Table 2. The presented studies were selected on the following criteria: prospective follow-up studies in cohorts comprising at least 100 patients with recent-onset RA (duration of symptoms 2 years or less, fulfilling ACR criteria for RA), including a multivariate analysis of baseline characteristics, and preferably a description of the accuracy of the prediction model, and published between 1997 and 2006.

As shown in the table, baseline radiographic score, presence of autoantibodies, and serologic evidence of disease activity are predictive of more severe destructive disease in most studies. Interestingly, an additional study by Goronzy and colleagues included several new genetic and immunologic markers to predict radiographic damage (122). However, multivariate analysis again revealed RF and erosive disease among the strongest predictors of damage, while none of the newer markers was selected as a strong predictor.

The variety in variables predicting worse functional ability is high, and accuracy is less than of those predicting radiographic damage. Functional ability at baseline seems to be the most consistent prognostic factor, followed by disease activity.

Among the markers predicting remission, disease activity and RF seem to be consistent. However, as shown in the right column, predicting future remission at baseline in the individual patient seems barely possible.

Outline of thesis

In this thesis, the effectiveness of different treatment strategies in early rheumatoid arthritis is evaluated. Tailor-made treatment of the individual patient with early RA is possible if both disease course and treatment response can be accurately predicted at the moment of presentation. Therefore, several clinical, serologic, and genetic markers are studied for their association with treatment response and/ or a more severe disease course.

Chapter 2 describes the accuracy of a prediction model, based on well-known prognostic markers, using data from the Early Arthritis Clinic (EAC) from the Leiden University Medical Center. This analysis explores the possibility to make reliable treatment decisions in early RA with currently available prognostics markers.
Chapter 3 reviews the use of TNF-α inhibitors in early RA.

In chapter 4 and 5, the primary outcomes of the BeSt study are presented. 'BeSt' is a Dutch acronym for treatment strategies: Behandelstrategieën. This multicenter randomized single-blinded clinical trial compared the efficacy of four different treatment strategies in early RA: 1. sequential monotherapy, where patients started with one DMARD, which was replaced by another in case of insufficient response, 2. step-up combination therapy, where patients also started with one DMARD, but where other DMARDs were added step by step in case of insufficient response, 3. initial combination therapy, where patients directly started with a combination of methotrexate, sulphasalazine, and a tapered high-dose of prednisolone, and 4. initial combination therapy, where patients started with a combination of methotrexate and infliximab, a TNF-α inhibitor.

Chapter 6 reports on the association between reduction of disease activity and radiographic and functional outcomes for different treatment strategies in the BeSt study.

In chapter 7, 8, and 9, different prognostics markers are evaluated based on data from the BeSt study. Chapter 7 describes the association between ex vivo LPS induced interleukine 1β and interleukine 1 receptor antagonist production with diagnosis and prognosis of RA. Chapter 8 describes the association between single nucleotide polymorphisms in genes encoding for folate cycle enzymes and efficacy and toxicity of methotrexate. Chapter 9 describes the association between presence of autoantibodies (RF and ACPA) and carriership of shared epitope with radiographic outcome for the different treatment strategies in the BeSt study. The results of the studies described in this thesis are summarized and discussed in chapter 10.

REFERENCES

1. Firestein GS. Evolving concepts of rheumatoid arthritis 64. Nature 2003; 423(6937):356-361.
2. Callahan LF, Pincus T. Mortality in the rheumatic diseases. Arthritis Care Res 1995; 8(4):229-241.
3. Abdel-Nasser AM, Rasker JJ, Valkenburg HA. Epidemiological and clinical aspects relating to the variability of rheumatoid arthritis. Semin Arthritis Rheum 1997; 27(2):123-140.
4. Landre-Beauvais AJ. The first description of rheumatoid arthritis. Unabridged text of the doctoral dissertation presented in 1800. Joint Bone Spine 2001; 68(2):130-143.
5. Ropes M, Bennett G, Cobb R, Jacox S, Jessar R. Proposed diagnostic criteria for rheumatoid arthritis; report of a study conducted by a committee of the American Rheumatism Association. J Chronic Dis 1957; 5(6):630-635.
6. Spector TD. Rheumatoid arthritis. Rheum Dis Clin North Am 1990; 16(3):513-537.
7. Van der Linden SJ. Reumatoide artritis samengevat. 2003. Bilthoven, RIVM. Volksgezondheid Toekomst Verkenning. Nationaal Kompas Volksgezondheid.
8. Roeding A. Reumatoide artritis 2001-2004. 2006. Bilthoven, RIVM. Volksgezondheid Toekomst Verkenning. Nationale Atlas Volksgezondheid.
9. Slobbe CCJ, Kommer GJ, Smit JM, Groen J, Meerding WJ, Pader JJ. Kosten van ziekten in Nederland 2003. ISBN 10 90 6 960 145 1. 2003. Bilthoven, RIVM. Kosten van ziekten in Nederland.
10. Symmons DP. Epidemiology of rheumatoid arthritis: determinants of onset, persistence and outcome. Best Pract Res Clin Rheumatol 2002; 16(5):707-722.
11. Lanzavecchia A. Receptor-mediated antigen uptake and its effect on antigen presentation to class II-restricted T lymphocytes. Annu Rev Immunol 1990; 8:773-793.
12. Takemura S, Klimiuk PA, Braun A, Goronzy JJ, Weyand CM. T cell activation in rheumatoid synovium is B cell dependent. J Immunol 2001; 167(8):4710-4718.
13. Waaler E. On the occurence of a factor in human serum activating the specific agglutination of sheep blood corpuscles. Acta Pathol Microbiol Scand 1940; 17(x):172-188.
14. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000; 43(1):155-163.
15. Matsumoto I, Lee DM, Goldbach-Mansky R, Sumida T, Hitchon CA, Schur PH et al. Low prevalence of antibodies to glucose-6-phosphate isomerase in patients with rheumatoid arthritis and a spectrum of other chronic autoimmune disorders. Arthritis Rheum 2003; 48(4):944-954.
16. Nienhuis R, Mandema EA. A new serum factor in patients with rheumatoid arthritis; the antiperinuclear factor. Ann Rheum Dis 1964; 23:302-305.
17. Skriner K, Sommergruber WH, Tremmel V, Fischer I, Barta A, Smolen JS et al. Anti-A2/RA33 autoantibodies are directed to the RNA binding region of the A2 protein of the heterogeneous nuclear ribonucleoprotein complex. Differential epitope recognition in rheumatoid arthritis, systemic lupus erythematosus, and mixed connective tissue disease. J Clin Invest 1997; 100(1):127-135.

18. Masson-Bessiere C, Sebbag M, Girbal-Neuhauser E, Nogueira L, Vincent C, Senshu T et al. The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the alpha- and beta-chains of fibrin. J Immunol 2001; 166(6):4177-4184.
19. Vossenaar ER, Nijenhuis S, Helsen MM, van der HA, Senshu T, van den Berg WB et al. Citrullination of synovial proteins in murine models of rheumatoid arthritis. Arthritis Rheum 2003; 48(9):2489-2500.
20. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH et al. Specific autoantibodies precede the symptons of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 2004; 50(2):380-386
21. van Gaalen FA, Visser H, Huizinga TW. A comparison of the diagnostic accuracy and prognostic value of the first and second anti-cyclic citrullinated peptides (CCP1 and CCP2) autoantibody tests for rheumatoid arthritis. Ann Rheum Dis 2005; 64(10):1510-1512.
22. Fox DA. The role of T cells in the immunopathogenesis of rheumatoid arthritis: new perspectives. Arthritis Rheum 1997; 40(4):598-609.
23. Sarkar S, Fox DA. Regulatory T cell defects in rheumatoid arthritis. Arthritis Rheum 2007; 56(3):710-713.
24. Stastny P. Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. N Engl J Med 1978; 298(16):869-871.
25. de Jongh BM, van Romunde LK, Valkenburg HA, de Lange GG, van Rood JJ. Epidemiological study of HLA and GM in rheumatoid arthritis and related symptoms in an open Dutch population. Ann Rheum Dis 1984; 43(4):613-619.
26. Woodrow JC, Nichol FE, Zaphiropoulos G. DR antigens and rheumatoid arthritis: a study of two populations. Br Med J (Clin Res Ed) 1981; 283(6302):1287-1288.
27. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987; 30(11):1205-1213.
28. Linn-Rasker SP, van der Helm-van Mil AH, van Gaalen FA, Kloppenburg M, de Vries RR, le Cessie S et al. Smoking is a risk factor for anti-CCP antibodies only in rheumatoid arthritis patients who carry HLA-DRB1 shared epitope alleles. Ann Rheum Dis 2006; 65(3):366-371.
29. Deighton CM, Wentzel J, Cavanagh G, Roberts DF, Walker DJ. Contribution of inherited factors to rheumatoid arthritis. Ann Rheum Dis 1992; 51(2):182-185.
30. MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K et al. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum 2000; 43(1):30-37.
31. Deighton CM, Walker DJ, Griffiths ID, Roberts DF. The contribution of HLA to rheumatoid arthritis. Clin Genet 1989; 36(3):178-182.
32. Crilly A, Maiden N, Capell HA, Madhok R. Predictive value of interleukin 1 gene polymorphisms for surgery. Ann Rheum Dis 2000; 59(9):695-699.
33. Martinez A, Fernandez-Arquero M, Pascual-Salcedo D, Conejero L, Alves H, Balsa A et al. Primary association of tumor necrosis factor-region genetic markers with susceptibility to rheumatoid arthritis. Arthritis Rheum 2000; 43(6):1366-1370.

34. Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. Annu Rev Immunol 1996; 14:397-440.
35. Firestein GS, Alvaro-Gracia JM, Maki R. Quantitative analysis of cytokine gene expression in rheumatoid arthritis. J Immunol 1990; 144(9):3347-3353.
36. Bresnihan B, Alvaro-Gracia JM, Cobby M, Doherty M, Domljan Z, Emery P et al. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. Arthritis Rheum 1998; 41(12):2196-2204.
37. Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS et al. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet 1994; 344(8930):1105-1110.
38. Moreland LW, Baumgartner SW, Schiff MH, Tindall EA, Fleischmann RM, Weaver AL et al. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. N Engl J Med 1997; 337(3):141-147.
39. van de Putte LB, Rau R, Breedveld FC, Kalden JR, Malaise MG, van Riel PL et al. Efficacy and safety of the fully human anti-tumour necrosis factor alpha monoclonal antibody adalimumab (D2E7) in DMARD refractory patients with rheumatoid arthritis: a 12 week, phase II study. Ann Rheum Dis 2003; 62(12):1168-1177.
40. Arnett FC, Edworthy SM, Bloch DA, Mcshane DJ, Fries JF, Cooper NS et al. The American Rheumatism Association 1987 revised criteria for the classification of Rheumatoid Arthritis. Arthritis and Rheumatism 1988; 31(3):315-324.
41. Kaarela K, Kauppi MJ, Lehtinen KE. The value of the ACR 1987 criteria in very early rheumatoid arthritis. Scand J Rheumatol 1995; 24(5):279-281.
42. Saraux A, Berthelot JM, Chales G, Le Henaff C, Thorel JB, Hoang S et al. Ability of the American College of Rheumatology 1987 criteria to predict rheumatoid arthritis in patients with early arthritis and classification of these patients two years later. Arthritis Rheum 2001; 44(11):2485-2491.
43. Verpoort KN, van Dongen H, Allaart CF, Toes RE, Breedveld FC, Huizinga TW. Undifferentiated arthritis-disease course assessed in several inception cohorts. Clin Exp Rheumatol 2004; 22(5 Suppl 35):S12-S17.
44. van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, de Jong BA, Breedveld FC, Verweij CL et al. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. Arthritis Rheum 2004; 50(3):709-715.
45. Visser H, le Cessie S, Vos K, Breedveld FC, Hazes JM. How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis. Arthritis Rheum 2002; 46(2):357-365.
46. Verpoort KN, van Gaalen FA, van der Helm-van Mil AH, Schreuder GM, Breedveld FC, Huizinga TW et al. Association of HLA-DR3 with anti-cyclic citrullinated peptide antibody-negative rheumatoid arthritis. Arthritis Rheum 2005; 52(10):3058-3062.
47. Huizinga TW, Machold KP, Breedveld FC, Lipsky PE, Smolen JS. Criteria for early rheumatoid arthritis: from Bayes' law revisited to new thoughts on pathogenesis. Arthritis Rheum 2002; 46(5):1155-1159.

48. Clark P, Casas E, Tugwell P, Medina C, Gheno C, Tenorio G et al. Hydroxychloroquine compared with placebo in rheumatoid arthritis. A randomized controlled trial. Ann Intern Med 1993; 119(11):1067-1071.
49. Epstein WV, Henke CJ, Yelin EH, Katz PP. Effect of parenterally administered gold therapy on the course of adult rheumatoid arthritis. Ann Intern Med 1991; 114(6):437-444.
50. Forre O. Radiologic evidence of disease modification in rheumatoid arthritis patients treated with cyclosporine. Results of a 48-week multicenter study comparing low-dose cyclosporine with placebo. Norwegian Arthritis Study Group. Arthritis Rheum 1994; 37(10):1506-1512.
51. Jeurissen ME, Boerbooms AM, van de Putte LB, Doesburg WH, Lemmens AM. Influence of methotrexate and azathioprine on radiologic progression in rheumatoid arthritis. A randomized, double-blind study. Ann Intern Med 1991; 114(12):999-1004.
52. Jones G, Halbert J, Crotty M, Shanahan EM, Batterham M, Ahern M. The effect of treatment on radiological progression in rheumatoid arthritis: a systematic review of randomized placebo-controlled trials. Rheumatology (Oxford) 2003; 42(1):6-13.
53. van der Heijde DM, van Riel PL, Nuver-Zwart IH, Gribnau FW, vad de Putte LB. Effects of hydroxychloroquine and sulphasalazine on progression of joint damage in rheumatoid arthritis. Lancet 1989; 1(8646):1036-1038.
54. van Everdingen AA, Jacobs JW, Siewertsz Van Reesema DR, Bijlsma JW. Low-dose prednisone therapy for patients with early active rheumatoid arthritis: clinical efficacy, disease-modifying properties, and side effects: a randomized, double-blind, placebo-controlled clinical trial. Ann Intern Med 2002; 136(1):1-12.
55. van der Heijde DM, van Leeuwen MA, van Riel PL, Koster AM, 't Hof MA, van Rijswijk MH et al. Biannual radiographic assessments of hands and feet in a three-year prospective followup of patients with early rheumatoid arthritis. Arthritis Rheum 1992; 35(1):26-34.
56. Lard LR, Visser H, Speyer I, vander Horst-Bruinsma IE, Zwinderman AH, Breedveld FC et al. Early versus delayed treatment in patients with recent-onset rheumatoid arthritis: comparison of two cohorts who received different treatment strategies. Am J Med 2001; 111(6):446-451.
57. van der Heide A, Jacobs JW, Bijlsma JW, Heurkens AH, Booma-Frankfort C, van der Veen MJ et al. The effectiveness of early treatment with "second-line" antirheumatic drugs. A randomized, controlled trial. Ann Intern Med 1996; 124(8):699-707.
58. Fries JF, Williams CA, Ramey DR, Bloch DA. The relative toxicity of alternative therapies for rheumatoid arthritis: implications for the therapeutic progression. Semin Arthritis Rheum 1993; 23(2 Suppl 1):68-73.
59. Verhoeven AC, Boers M, Tugwell P. Combination therapy in rheumatoid arthritis - updated systematic review. Br J Rheumatol 1998;37:612-9.
60. Calguneri M, Pay S, Caliskaner Z, Apras S, Kiraz S, Ertenli I et al. Combination therapy versus monotherapy for the treatment of patients with rheumatoid arthritis. Clin Exp Rheumatol 1999; 17(6):699-704.
61. van Gestel AM, Haagsma CJ, Furst DE, van Riel PL. Treatment of early rheumatoid arthritis patients with slow-acting anti-rheumatic drugs (SAARDs). Baillieres Clin Rheumatol 1997; 11(1):65-82.

62. Pincus T, O'dell JR, Kremer JM. Combination therapy with multiple disease-modifying antirheumatic drugs in rheumatoid arthritis: a preventive strategy. Ann Intern Med 1999; 131(10):768-774.
63. Tugwell P, Pincus T, Yocum D, Stein M, Gluck O, Kraag G et al. Combination therapy with cyclosporine and methotrexate in severe rheumatoid arthritis. The Methotrexate-Cyclosporine Combination Study Group. N Engl J Med 1995; 333(3):137-141.
64. O'Dell JR, Haire CE, Erikson N, Drymalski W, Palmer W, Eckhoff PJ et al. Treatment of rheumatoid arthritis with methotrexate alone, sulfasalazine and hydroxychloroquine, or a combination of all three medications. N Engl J Med 1996; 334(20):1287-1291.
65. Boers M, Verhoeven AC, Markusse HM, van de Laar MA, Westhovens R, van Denderen JC et al. Randomised comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis. Lancet 1997; 350(9074):309-318.
66. Landewe RB, Boers M, Verhoeven AC, Westhovens R, van de Laar MA, Markusse HM et al. COBRA combination therapy in patients with early rheumatoid arthritis: long-term structural benefits of a brief intervention. Arthritis Rheum 2002; 46(2):347-356.
67. Shanahan JC, Moreland LW, Carter RH. Upcoming biologic agents for the treatment of rheumatic diseases. Curr Opin Rheumatol 2003; 15(3):226-236.
68. Keystone EC, Kavanaugh AF, Sharp JT, Tannenbaum H, Hua Y, Teoh LS et al. Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial. Arthritis Rheum 2004; 50(5):1400-1411.
69. Klareskog L, van der Heijde D., de Jager JP, Gough A, Kalden J, Malaise M et al. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. Lancet 2004; 363(9410):675-681.
70. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. N Engl J Med 2000; 343(22):1594-1602.
71. de Asit N, Le L, X, Czernichow P, Bertho JM, Delcambre B, Tonnel F et al. How do French rheumatologists treat early rheumatoid arthritis? Rev Rhum Engl Ed 1996; 63(3):188-195.
72. Galindo-Rodriguez G, Avina-Zubieta JA, Fitzgerald A, LeClerq SA, Russell AS, Suarez-Almazor ME. Variations and trends in the prescription of initial second line therapy for patients with rheumatoid arthritis. J Rheumatol 1997; 24(4):633-638.
73. Suarez-Almazor ME, Soskolne CL, Saunders LD, Russell AS. Use of second line drugs for the treatment of rheumatoid arthritis in Edmonton, Alberta. Patterns of prescription and longterm effectiveness. J Rheumatol 1995; 22(5):836-843.
74. Aletaha D, Smolen JS. The rheumatoid arthritis patient in the clinic: comparing more than 1,300 consecutive DMARD courses. Rheumatology (Oxford) 2002; 41(12):1367-1374.

75. Maetzel A, Wong A, Strand V, Tugwell P, Wells G, Bombardier C. Meta-analysis of treatment termination rates among rheumatoid arthritis patients receiving disease-modifying anti-rheumatic drugs. Rheumatology (Oxford) 2000; 39(9):975-981.
76. Breedveld FC, Weisman MH, Kavanaugh AF, Cohen SB, Pavelka K, van Vollenhoven R et al. The PREMIER study: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. Arthritis Rheum 2006; 54(1):26-37.
77. Klareskog L, van der Heijde DM, de Jager JP, Gough A, Kalden J, Malaise M et al. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. Lancet 2004; 363(9410):675-681.
78. St Clair EW, van der Heijde DM, Smolen JS, Maini RN, Bathon JM, Emery P et al. Combination of infliximab and methotrexate therapy for early rheumatoid arthritis: a randomized, controlled trial. Arthritis Rheum 2004; 50(11):3432-3443.
79. van der Heijde DM, Klareskog L, Rodriguez-Valverde V, Codreanu C, Bolosiu H, Melo-Gomes J et al. Comparison of etanercept and methotrexate, alone and combined, in the treatment of rheumatoid arthritis: two-year clinical and radiographic results from the TEMPO study, a double-blind, randomized trial. Arthritis Rheum 2006; 54(4):1063-1074.
80. Matteson EL, Weyand CM, Fulbright JW, Christianson TJ, McClelland RL, Goronzy JJ. How aggressive should initial therapy for rheumatoid arthritis be? Factors associated with response to 'non-aggressive' DMARD treatment and perspective from a 2-yr open label trial. Rheumatology (Oxford) 2004; 43(5):619-625.
81. O'Dell JR. Treating rheumatoid arthritis early: a window of opportunity? Arthritis Rheum 2002; 46(2):283-285.
82. Boers M, Tugwell P, Felson DT, van Riel PL, Kirwan JR, Edmonds JP et al. World Health Organization and International League of Associations for Rheumatology core endpoints for symptom modifying antirheumatic drugs in rheumatoid arthritis clinical trials. J Rheumatol Suppl 1994; 41:86-89.
83. Felson DT, Anderson JJ, Boers M, Bombardier C, Chernoff M, Fried B et al. The American College of Rheumatology preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. The Committee on Outcome Measures in Rheumatoid Arthritis Clinical Trials. Arthritis Rheum 1993; 36(6):729-740.
84. Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C et al. American College of Rheumatology preliminary definition of improvement in Rheumatoid Arthritis. Arthritis and Rheumatism 1995; 38(6):727-735.
85. van der Heijde DM, van 't Hof M, van Riel PL, van de Putte LB. Development of a Disease Activity score based on judgement in clinical practice by rheumatologists. J Rheum 1993; 20(3):579-581
86. Fuchs HA. The use of the disease activity score in the analysis of clinical trials in rheumatoid arthritis. J Rheumatol 1993; 20(11):1863-1866.

87. van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European league Against Rheumatism responce criteria for rheumatoid arthritis-comparison with the preliminary American college of Rheumatology and the World Health Organization International league Against Rheumatism criteria. Arthritis Rheum 1996; 39(1):34-40.
88. Prevoo ML, van Gestel AM, van 't Hof MA, van Rijswijk MH, van de Putte LBA, van Riel PLCM. Remission in a prospective study of patients with rheumatoid arthritis. American rheumatism association preliminary remission criteria in relation to the disease activity score. British Journal of Rheumatology 1996; 35(11):1101-1105.
89. Prevoo ML, 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995; 38(1):44-48.
90. Grigor C, Capell H, Stirling A, McMahon AD, Lock P, Vallance R et al. Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomised controlled trial. Lancet 2004; 364(9430):263-269.
91. Fries JF, Spitz P, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. Arthritis Rheum 1980; 23(2):137-145.
92. Siegert CE, Vleming LJ, Vandenbroucke JP, Cats A. Measurement of Disability in Dutch Rheumatoid-Arthritis Patients. Clinical Rheumatology 1984; 3(3):305-309.
93. Drossaers-Bakker KW, de Buck M, van Zeben D, Zwinderman AH, Breedveld FC, Hazes JM. Long-term course and outcome of functional capacity in rheumatoid arthritis: the effect of disease activity and radiologic damage over time. Arthritis Rheum 1999; 42(9):1854-1860.
94. Wells GA, Tugwell P, Kraag GR, Baker PR, Groh J, Redelmeier DA. Minimum important difference between patients with rheumatoid arthritis: the patient's perspective. J Rheumatol 1993; 20(3):557-560.
95. Drossaers-Bakker KW, Zwinderman AH, Vlieland TP, van Zeben D, Vos K, Breedveld FC et al. Long-term outcome in rheumatoid arthritis: a simple algorithm of baseline parameters can predict radiographic damage, disability, and disease course at 12-year followup. Arthritis Rheum 2002; 47(4):383-390.
96. Scott DL, Pugner K, Kaarela K, Doyle DV, Woolf A, Holmes J et al. The links between joint damage and disability in rheumatoid arthritis. Rheumatology (Oxford) 2000; 39(2):122-132.
97. Graudal NA, Jurik AG, de Carvalho A, Graudal HK. Radiographic progression in rheumatoid arthritis: a long-term prospective study of 109 patients. Arthritis Rheum 1998; 41(8):1470-1480.
98. Hulsmans HM, Jacobs JW, van der Heijde DM, Albada-Kuipers GA, Schenk Y, Bijlsma JW. The course of radiologic damage during the first six years of rheumatoid arthritis. Arthritis Rheum 2000; 43(9):1927-1940.
99. Kirwan JR. The relationship between synovitis and erosions in rheumatoid arthritis. Br J Rheumatol 1997; 36(2):225-228.
100. Mulherin D, Fitzgerald O, Bresnihan B. Clinical improvement and radiological deterioration in rheumatoid arthritis: evidence that the pathogenesis of synovial inflammation and articular erosion may differ. Br J Rheumatol 1996; 35(12):1263-1268.

101. Landewe R, van der HD, Klareskog L, van Vollenhoven R, Fatenejad S. Disconnect between inflammation and joint destruction after treatment with etanercept plus methotrexate: results from the trial of etanercept and methotrexate with radiographic and patient outcomes. Arthritis Rheum 2006; 54(10):3119-3125.
102. van den Berg WB, van Riel PL. Uncoupling of inflammation and destruction in rheumatoid arthritis: myth or reality? Arthritis Rheum 2005; 52(4):995-999.
103. Molenaar ET, Voskuyl AE, Dinant HJ, Bezemer PD, Boers M, Dijkmans BA. Progression of radiologic damage in patients with rheumatoid arthritis in clinical remission. Arthritis Rheum 2004; 50(1):36-42.
104. Boini S, Guillemin F. Radiographic scoring methods as outcome measures in rheumatoid arthritis: properties and advantages. Ann Rheum Dis 2001; 60(9):817-827.
105. van der Heijde DM. How to read radiographs according to the Sharp/van der Heijde method. Journal of Rheumatology 1999; 26(3):743-745.
106. van der Heijde DM, van Leeuwen MA, van Riel PL, van de Putte LB. Radiographic progression on radiographs of hands and feet during the first 3 years of rheumatoid arthritis measured according to Sharp's method (van der Heijde modification). J Rheumatol 1995; 22(9):1792-1796.
107. Boini S, Guillemin F. Radiographic scoring methods as outcome measures in rheumatoid arthritis: properties and advantages. Ann Rheum Dis 2001; 60(9):817-827.
108. Bruynesteyn K, van der HD, Boers M, Saudan A, Peloso P, Paulus H et al. Detecting radiological changes in rheumatoid arthritis that are considered important by clinical experts: influence of reading with or without known sequence. J Rheumatol 2002; 29(11):2306-2312.
109. Young A, van der Heijde DM. Can we predict aggressive disease? Baillieres Clin Rheumatol 1997; 11(1):27-48.
110. van Zeben D, Hazes JM, Zwinderman AH, Vandenbroucke JP, Breedveld FC. Factors predicting outcome of rheumatoid arthritis: results of a followup study. J Rheumatol 1993; 20(8):1288-1296.
111. Combe B, Dougados M, Goupille P, Cantagrel A, Eliaou JF, Sibilia J et al. Prognostic factors for radiographic damage in early rheumatoid arthritis: a multiparameter prospective study. Arthritis Rheum 2001; 44(8):1736-1743.
112. Lindqvist E, Jonsson K, Saxne T, Eberhardt K. Course of radiographic damage over 10 years in a cohort with early rheumatoid arthritis. Ann Rheum Dis 2003; 62(7):611-616.
113. Forslind K, Ahlmen M, Eberhardt K, Hafstrom I, Svensson B. Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). Ann Rheum Dis 2004; 63(9):1090-1095.
114. Dixey J, Solymossy C, Young A. Is it possible to predict radiological damage in early rheumatoid arthritis (RA)? A report on the occurrence, progression, and prognostic factors of radiological erosions over the first 3 years in 866 patients from the Early RA Study (ERAS). J Rheumatol Suppl 2004; 69:48-54.
115. Lindqvist E, Eberhardt K, Bendtzen K, Heinegard D, Saxne T. Prognostic laboratory markers of joint damage in rheumatoid arthritis. Ann Rheum Dis 2005; 64(2):196-201.
116. Lindqvist E, Saxne T, Geborek P, Eberhardt K. Ten year outcome in a cohort of patients with early rheumatoid arthritis: health status, disease process, and damage. Ann Rheum Dis 2002; 61(12):1055-1059.

117. Bansback N, Young A, Brennan A, Dixey J. A prognostic model for functional outcome in early rheumatoid arthritis. J Rheumatol 2006; 33(8):1503-1510.
118. Combe B, Cantagrel A, Goupille P, Bozonnat MC, Sibilia J, Eliaou JF et al. Predictive factors of 5-year health assessment questionnaire disability in early rheumatoid arthritis. J Rheumatol 2003; 30(11):2344-2349.
119. Gossec L, Dougados M, Goupille P, Cantagrel A, Sibilia J, Meyer O et al. Prognostic factors for remission in early rheumatoid arthritis: a multiparameter prospective study. Ann Rheum Dis 2004; 63(6):675-680.
120. Forslind K, Hafstrom I, Ahlmen M, Svensson B. Sex: a major predictor of remission in early rheumatoid arthritis? Ann Rheum Dis 2007; 66(1):46-52.
121. Eberhardt K, Fex E. Clinical course and remission rate in patients with early rheumatoid arthritis: relationship to outcome after 5 years. Br J Rheumatol 1998; 37(12):1324-1329.
122. Goronzy JJ, Matteson EL, Fulbright JW, Warrington KJ, Chang-Miller A, Hunder GG et al. Prognostic markers of radiographic progression in early rheumatoid arthritis. Arthritis Rheum 2004; 50(1):43-54.

Chapter 2

Using predicted disease outcome to provide differentiated treatment of early rheumatoid arthritis

J. K. de Vries-Bouwstra
S. Le Cessie
C. F. Allaart
F. C. Breedveld
T. W. J. Huizinga

J Rheumatol 2006; 33(9): 1747-53.

ABSTRACT

Objective To determine the usefulness of a prediction model for making treatment decisions in early rheumatoid arthritis (RA).

Methods In 152 patients with early RA, progression of radiographic damage during the first year (Sharp-van der Heijde [SH] score > 0) was assessed and used to define actual disease outcome. Available variables at baseline were entered in a multivariate regression analysis with progression score as dependent variable. This model was used to predict disease outcome in every patient. Using the standard deviations of the predicted disease outcome, patients were divided into three groups: (1) severe disease: high probability (≥ 0.8) for progression > 0, (2) mild disease: high probability (≥ 0.8) for progression ≤ 0, and (3) not classified: no high probability for either option. It was determined how many patients could be classified by using this model.

Results One hundred nine patients (71.7 %) showed joint damage progression during the first year. Baseline variables available were: age, sex, duration of symptoms, duration of morning stiffness, patient's global assessment of disease activity, Health Assessment Questionnaire -score, swollen and painful joint count, bilateral compression pain in metatarsophalangeals, rheumatoid factor positivity, erythrocyte sedimentation rate, shared epitope positivity, SH-score, and the presence of erosions. The R^2 value (≈ variation explained) of the prediction model was 0.36. By using this model 46.3% of patients could be classified as having severe disease, 0 % as having mild disease, and 53.7% could not be classified.

Conclusion To be able to make treatment decisions in early RA based on predicted disease outcome, a better prediction of disease outcome is needed, making the search for better prognostic variables urgent.

INTRODUCTION

Over the last decade several studies have shown that early treatment of rheumatoid arthritis (RA) with disease modifying antirheumatic drugs (DMARDs) results in more effective suppression of disease activity and substantial reduction of joint damage (1-3). Even a brief delay of 3-9 months to start of DMARD therapy already has significant negative effect on radiographic outcome after 2 years (4,5). Based on these observations DMARD therapy currently is started as soon as a patient is diagnosed with RA.

This early start poses problems. It has been shown that early, aggressive intervention of RA with a combination of DMARDs can modify long-term disease outcome independent of the treatment in the following years (6,7). This indicates that there is a period very early in the course of RA during which radiographic progression rates can be 'reset' and therapeutic interventions can actually modify disease outcome. On the other hand, good clinical responses and prevention of progression of joint damage can be achieved with early DMARD monotherapy in a substantial proportion of patients. The results of the ERA trial, comparing etanercept and methotrexate (MTX) in patients with early RA, showed that among patients who received MTX monotherapy 60% had no increase of erosions, compared with 72% of patients who received etanercept 25 mg (P = 0.007) (8). These observations are confirmed by the results of the BeSt study, a randomized, single-blinded clinical trial comparing 4 different treatment strategies in early RA. To achieve low disease activity, treatment was adjusted less often in the groups treated more aggressively with combination therapy including either prednisone or infliximab. However, in the group that started with monotherapy, persistent low disease activity could be maintained in 33% of patients over a period of 2 years with MTX monotherapy (9).

Thus, although there is strong evidence for starting treatment early in the course of RA, these results also indicate that to achieve good clinical and radiographic responses most aggressive strategies are not necessary for all patients. Supposing that patients with relatively mild RA need less aggressive and less toxic treatment strategies than patients with severe erosive disease, both subsets of patients should be identified at the moment of diagnosis. This way both subsets of patients will directly receive appropriate treatment with respect to expected disease outcome: an individual, 'tailor-made' treatment strategy. In order to provide a tailor-made treatment strategy clinicians should ideally have at their disposal a set of baseline variables that accurately predicts disease outcome for every individual patient presenting with RA.

Of all aspects of RA, those resulting in physical impairment, such as joint damage and cumulative disease activity, are particularly specific for the disease and are highly associated with functional ability. Joint damage, disease activity, and functional ability are all used as outcome indicators (10,11). Of these, joint damage is determined objectively and represents the cumulative effects of the disease on the joints at any point in time (11). Several studies investigating factors predicting radiographic progression have shown that a combination of predicting factors can correctly identify up to 83% of patients with

progressive erosive disease (12). The question is whether and to what extent one can rely on (a combination of) these prognostic factors when addressing therapeutic questions in the individual patient with RA. Predictive variables have to meet stringent requirements for use in the prospective situation since by definition these variables are identified in other cohorts. Apart from correctly identifying a group of patients with severe disease retrospectively, a prediction model should guarantee a clinician enough certainty about the predicted disease outcome in the individual patient presenting with RA to make therapeutic decisions.

The aim of our study was to evaluate the usefulness of a prediction model for making treatment decisions in early RA. Aggressive treatment was defined as directly starting with a combination of DMARDs, including MTX and either a high dose prednisone or a tumor necrosis factor (TNF)-α inhibitor. We defined disease outcome by the presence (severe RA) or absence (mild RA) of joint damage progression. The main goal was to predict disease outcome in every individual patient as accurately as possible with all available predictive variables. In order to rely on a prediction model for therapeutic decisions, it was assumed that a clinician should have at least 80% certainty about the predicted disease outcome in the individual patient. With this assumption, the percentage of patients with early RA that could be classified as probably having severe disease or as probably having mild disease was evaluated.

PATIENTS AND METHODS

Patients

Patients were selected from the Early Arthritis Clinic (EAC) of the Leiden University Medical Center (LUMC). The EAC was started in 1993 at the Department of Rheumatology of the LUMC, the only rheumatology clinic for rheumatic disease patients in an area with 300,000 inhabitants. The general practitioners in the area were encouraged to refer patients to the EAC if at least 2 of the following 3 features were present: joint pain, joint swelling, or reduced joint mobility. All patients were offered an appointment within 2 weeks of referral. The patients were included in the EAC if arthritis was confirmed by a rheumatologist, the duration of symptoms was < 2 years, and the patient had not seen a rheumatologist elsewhere for the same problem.

For our analysis a group of patients with early RA was selected that was not treated aggressively, ie, starting with DMARD monotherapy, to evaluate whether a prediction model could reliably identify those patients who would develop progressive disease without aggressive initial treatment. Therefore we selected, from the patients included in the EAC between 1993 and 1999, all patients who presented at the outpatient clinic with RA or with probable RA and in whom diagnosis of RA was confirmed at 3 months

after presentation. Patients had to have at least one year of follow-up and radiographs of hands and feet at baseline and after one year had to be available. Twelve percent of the EAC cohort fulfilled these criteria (n = 152). Definite RA was defined by the criteria of the American College of Rheumatology (13), but without the criterion that the symptoms must be of 6 weeks' duration, and observed by a physician. Of the included patients 85 % received DMARD therapy during the first year after diagnosis (starting with: chloroquine [40%], sulphasalazine (SSZ) [30%], or other drugs [30%]). Of all patients, 14 (9.2%) received a combination of DMARDs during the first year, most (n = 9) a combination of an antimalarial drug with another DMARD (n = 3 antimalarial and MTX, n = 5 antimalarial and cyclosporine, n = 1 antimalarial and experimental peptide vaccination strategy). Other combinations used were: SSZ and low-dose prednisone (n = 2), MTX and low-dose prednisone (n = 2), and SSZ and interferon-β (n = 1). A combination of DMARDs was started after a mean period of 24 weeks after presentation. No patient was treated with a combination of drugs directly after presentation.

Baseline and follow-up assessments

At the first visit a standard diagnostic evaluation was performed according to the EAC protocol. All available potentially prognostic variables (10-12) from the patient's history, physical, and laboratory examination were used for the current analysis: age, sex, duration of symptoms at presentation, duration of morning stiffness, Health Assessment Questionnaire (HAQ) score, patient's assessment of disease activity, Ritchie Articular Index score (14), total swollen joint count, bilateral compression pain in the metatarsophalangeal (MTP) joints, erythrocyte sedimentation rate (ESR), IgM rheumatoid factor (IgM RF) positivity, shared epitope (SE) hetero- or homozygosity, radiographic damage according to Sharp-van der Heijde (SH), and the presence of erosions on radiographs.

For the presence of arthritis 54 joints were assessed. Maximum swollen joint count was 22 since the proximal and distal interphalangeal joints, metacarpophalangeal (MCP) joints, and the MTP joints were each scored as one joint (ie, one left and one right). The presence of IgM RF was measured at study entry by ELISA. Every value ≥ 5 units was considered positive. DNA isolation and HLA DQ and DR typing were performed at study entry. Radiographs of hands and feet taken at study entry and after one year follow-up were scored in random order for the presence of erosions and narrowing by an experienced rheumatologist blinded for clinical data. The reported scores are the SH total damage scores (15,16). Disease outcome after one year was defined by progression of radiographic damage, determined as the difference between the SH-score at one year and baseline. In order to evaluate currently available variables predicting disease outcome, 2 subsets of patients were identified: (1) *patients with severe disease:* patients with progression of radiographic damage (progression score > 0), and (2) *patients with mild disease:* patients without progression of radiographic damage (progression score ≤ 0).

Statistical analysis

Regression analysis A regression analysis was performed to predict disease outcome for every individual patient. As we explicitly preferred to predict progression of radiographic damage for every individual patient as accurate as possible, we performed linear regression with progression of joint damage (continuous) as dependent variable. Both continuous variables (age, visual analog scale, disease activity, duration of morning stiffness, swollen joint count, Ritchie, ESR, HAQ, SH-score) and categorical variables (duration of symptoms > or ≤ 6 weeks, IgM RF positivity, SE, bilateral compression pain MTP, and presence of erosions) were entered as covariates. Univariate as well as multivariate linear regression were performed to determine the strength of correlation of the baseline variables with disease outcome. After entering the baseline variables, it was tested if the DMARD therapy given did have additional effect on the outcome. The multivariate linear regression was used to predict disease outcome after one year for all individual patients. The positive predictive value (PPV) for mild versus severe disease was calculated. The R^2 (≈ variation explained) was calculated. With a sample size of 152 persons, given $\alpha = 0.05$ and with a power of 90%, each predictive variable with a correlation ≥ 0.26 with the dependent variable was detected ($R^2 = 0.07$). For each patient, the predicted progression score and the predicted standard deviation, both obtained from the multivariate linear regression analysis, were used to calculate the predicted probability for severe disease (progression score > 0).

Prediction model In order to rely on a prediction model for therapeutic decisions it was assumed that a clinician should have at least 80% certainty about the predicted disease outcome in the individual patient. Only those patients for whom 80% certainty about the predicted outcome could be guaranteed (only those patients for whom 80% of the standard deviation was > 0 [high probability for severe disease] and those patients for whom 80% of the standard deviation was < 0 [high probability for mild disease]) were identified as classifiable patients. Consequently 3 groups of patients were defined: (1) *probably severe disease:* high predicted probability (≥ 0.8) for progression of radiological damage (progression score > 0); (2) *probably mild disease:* high predicted probability (≥ 0.8) for absence of progression of radiological damage (progression score ≤ 0); and (3) *not classifiable:* predicted probability for severe disease between 0.2 and 0.8. In order to evaluate the effect of the prediction model on therapeutic decision-making it was calculated which percentage of patients was in each of the defined groups.

Hypothetical prediction model We also studied how the percentage of unclassifiable patients changed with the R^2 of a model. We started with the assumptions that the predicted score and the actual score were bivariate normally distributed, with the mean chosen such that the percentage of patients with progression was equal to the percentage in our dataset. Under these assumptions, for different values of R the expected percentage of patients in the 3 groups mentioned as above (probably severe disease, probably mild disease, and not classifiable), could be calculated analytically. It was determined which R^2 value is needed to obtain a prediction model with < 20% of patients in the group not classifiable.

RESULTS

Table 1. Baseline characteristics of patients with early RA (n = 152)

Median age (IQR), yrs	66 (55–76)
No. (%) female	103 (68)
Median symptom duration at first visit (IQR), wks	22 (11–45)
No. (%) IgM rheumatoid factor positive	93 (61)
Median no. swollen joints (IQR)	6 (4-8)
No. (%) patients with erosions in hands or feet	57 (38)
HLA-DRB1 shared epitope (SE) no. (%)*	
SE +/+	31 (22)
SE +/-	65 (46)
SE -/-	45 (32)

IQR = Interquartile range
* Available from 141 patients

Table 2. Radiographic results

	Baseline	One year follow-up
No. (%) with erosions	57 (37.5)	104 (68.4)
Sharp-van der Heijde score, median (IQR)	1 (0-4)	8 (1-18)
Progression of Sharp-van der Heijde score, median (IQR)	-	4 (0-14)
No. with progression of radiographic damage (%)	-	109 (71.7)

IQR = Interquartile range

Description of the cohort

The baseline characteristics of the 152 patients in the study are shown in Table 1. The major radiographic findings are shown in Table 2. Fifty-seven patients (38 %) presented with erosive damage at baseline. During the first year of follow-up another 48 patients developed erosive damage. Of all patients, 109 (71.7%) showed progression of radiographic damage during the first year. Median progression of the SH-score was 4 (interquartile range 0-14).

Table 3 shows the results of the univariate and multivariate linear regression analysis of all the available baseline variables. Of all baseline variables, the following had significant correlation with progression of radiographic damage in the univariate analysis: patient's global assessment of disease activity, total swollen joint count, Ritchie score, IgM RF positivity, ESR, SH-score, and presence of erosions. Total swollen joint count and IgM RF positivity both had significant correlation with progression of radiographic damage in the multivariate analysis. If the duration of symptoms at presentation exceeded 6 weeks, the progression score increased with 9.4 points ($P = 0.06$). In total 11 patients (12%) had a symptom duration < 6 weeks.

Patients who received no DMARD were compared with the patients who received a combination of drugs and the patients who were treated with one DMARD at a time. Of all variables used in the prediction model, a significant difference was found only for the proportion of patients positive for IgM RF: of patients who did not receive a DMARD 28% were IgM RF positive, of patients who received one DMARD at a time 67% were IgM RF positive, and of patients who received a combination 72% were IgM RF positive. Patients did not differ significantly for progression scores during the first year (P = 0.493), although median progression was slightly higher for those who received DMARD monotherapy (6.5) compared with those who received combination therapy (3.5) and those who did not receive DMARDs (2.0). Since the observed difference could be based on confounding by indication, it was analyzed whether treatment as defined above (none/one DMARD/combination therapy) contributed significantly to joint damage progression in a regression analysis, but no significant association was found (unstandardized regression coefficient [URC] 0.034; P = 0.934). In a multivariate analysis with IgM RF and treatment (none/one DMARD/combination), IgM RF was still significantly contributing to joint damage progression, and treatment was not (URC treatment 0.377, P = 0.344; URC IgM RF 11.6, P < 0.001). In line with this, treatment did not significantly change the outcome of the multivariate regression analysis (data not shown).

Table 3. Regression analysis of baseline variables

Baseline variables	Univariate analysis		Multiple regression analysis; R^2 = 0.363 (n = 95$^{\phi}$)	
	URC‡	*P*	URC‡	*P*
Age	- 0.1	0.568	- 0.1	0.390
Sex	2.1	0.488	1.2	0.715
Duration of symptoms at presentation > 6 weeks	6.2	0.129	9.4	0.060
VAS for disease activity †	1.1	0.032	0.9	0.123
Duration of morning stiffness	0.0	0.588	0.0	0.982
Total swollen joint count	1.3	0.005	1.7	0.010
Ritchie score	0.4	0.025	- 0.0	0.893
Bilateral compression pain MTP	4.0	0.161	6.0	0.086
IgM RF positivity	11.0	<0.001	10.2	0.003
ESR	0.1	0.028	0.1	0.277
Shared epitope positive $^{\lozenge}$	5.1	0.120	0.7	0.835
HAQ $^{\bullet}$	- 0.2	0.933	- 3.4	0.207
Sharp-van der Heijde score	0.4	0.006	0.3	0.312
Presence of erosions	7.2	0.015	1.2	0.974

$^{\phi}$ n = 95; patients for whom all variables are available; ‡ The unstandardized regression coefficient (URC) describes the amount of increase in progression of joint damage per unit of the baseline variable; ie, one more swollen joint at baseline accounts for 1.3 points in progression score during the first year; † available for 105 patients; $^{\lozenge}$ available for 141 patients; $^{\bullet}$ available for 130 patients. VAS: visual analog scale; MTP: metatarsophalangeal; ESR: erythrocyte sedimentation rate; HAQ: Health Assessment Questionnaire.

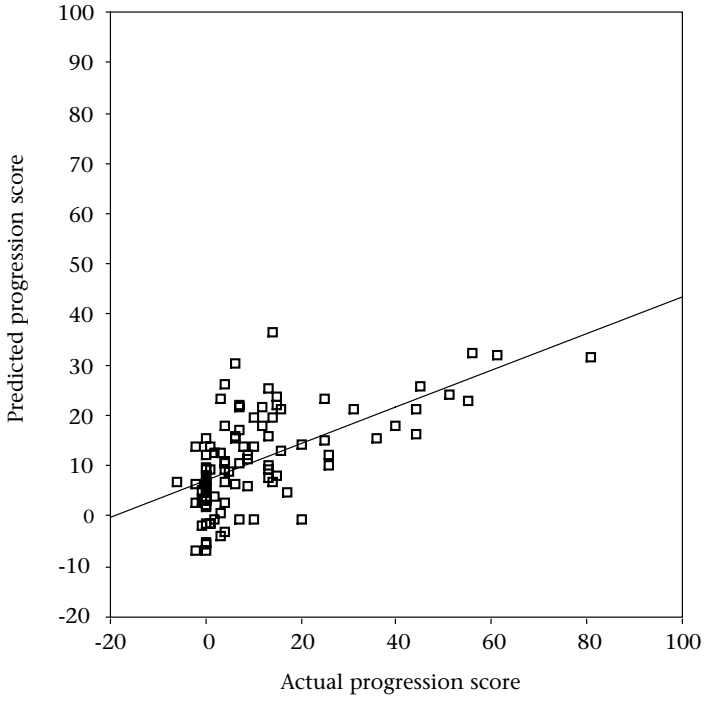


Figure 1. Actual versus predicted Sharp -van der Heijde score for progression of radiographic damage for patients for whom all variables were available (n = 95)

Prediction of radiographic damage

With the multivariate model, presence of progression of radiographic damage turned out to be predicted correctly in 75.3% of patients (PPV). The R^2 value of the multivariate regression analysis was 0.363 (R = 0.602). Patients for whom all variables were available (n = 95) were not significantly different in baseline characteristics from the total cohort except for HAQ score (mean value 1.06 for patients with all variables available vs 1.3 for patients with some variables missing; $P = 0.03$).

The multivariate regression analysis was used to predict disease outcome (ie, progression score), yielding an individual predicted progression score for every patient and the individual predicted probability on radiographic damage (progression > 0). Figure 1 shows the predicted progression scores versus the actual progression scores for the 95 patients for whom all variables were available. The histogram of residuals (predicted progression score minus actual progression score) was approximately normally distributed.

Next, the 95 patients were rearranged in 3 groups based on the predicted probability of radiographic damage (probably severe disease, probably mild disease, not classifiable; Table 4). As shown in Table 4, 46.3% of patients could be classified as probably having severe disease, 0 % as probably having mild disease, and 53.7% was not classifiable (a probability between 0.2 and 0.8). Apparently, the currently available predictive factors have better potential in identifying patients with severe disease compared to patients with mild disease: of 26 patients without actual progression of radiological damage only 6 had a predicted progression score ≤ 0.

Table 4. Evaluation of accuracy of prognostic model based on EAC cohort in classification of patients as having mild or severe disease; n = 95; r = 0.602, R^2 = 0.363, positive predictive value 75.3%

Classification by prediction model[ϕ]	Actual disease outcome:		Total No. classified (%)	Total No. classified correctly (%)	Total No. not classified (%)
	Mild disease	Severe disease			
	26	69			
Mild disease	0	0	0 (0)	0 (0)	
Severe disease	3	41	44 (46)	41 (43)	
Not classified	23	28			51 (54)

[ϕ] Classification when taking into account that 80% certainty about predicted outcome is needed for therapeutic decisions. EAC: Early Arthritis Clinic.

Table 5. Evaluation of accuracy of hypothetic prognostic models; percentage of patients classified as having either mild or severe disease

R^2 of hypothetical model	Classification by hypothetical prediction model:	Actual disease outcome:	
		Mild disease (28%)	Severe disease (72%)
R^2 = 0.49	Mild disease	9 %	3 %
	Severe disease	4 %	45 %
	Not classified	15 %	24 %
R^2 = 0.64	Mild disease	12 %	2 %
	Severe disease	3 %	50 %
	Not classified	13 %	20 %
R^2 = 0.9	Mild disease	19 %	1 %
	Severe disease	1 %	61 %
	Not classified	8 %	10 %

Hypothetical prediction model

Finally, the relation between the R^2 of a model and the percentage of patients not classifiable is shown in Table 5. In the case of R = 0.95 (R^2 = 0.9) in total 82% could be classified as having probably mild or severe disease. As shown by the data it was possible to identify patients with mild disease under the assumption of normally distributed actual and predicted progression scores.

DISCUSSION

A prediction model that could accurately identify subsets of patients with mild or severe disease in the early phase of RA would allow a clinician to make a conscious choice between different treatment strategies at the moment that DMARD therapy is started. Our main finding is that currently available prognostic variables in early RA are not sufficient to supply 'tailor-made treatment' of RA in the majority of patients. If the power of the prediction model is optimized in a hypothetical situation (R = 0.95), differentiated

therapeutic decisions would be possible in up to 82 % of patients, validating the search for more and better prognostic variables.

A drawback of our current study is that the prediction model was developed retrospectively and that it was not validated in another – ultimately prospective – cohort, possibly introducing bias in the identification of the prognostic variables. However, we chose to use all available and possible prognostic baseline variables without any selection of variables. The baseline variables used in the regression analysis have been indicated as predictive factors for outcome of RA in many studies. The variables that correlated most strongly with disease outcome (IgM RF positivity, swollen joint count, and disease duration) have also been identified as strong predictors of disease outcome by others (10,11,17-21), implying that selection bias most likely is not a major problem. In theory, bias may have occurred since the current analysis was carried out in a Caucasian population derived from one geographic area, but we are not aware of a specific phenotype of RA or specific risk factors in the population under study.

As shown in Table 4, the currently available predictive factors have better potential in identifying patients with severe disease compared to patients with mild disease. Twenty out of the 26 patients with actual mild disease (absence of progression of radiographic damage) had a predicted progression > 0. Of these 20 patients, baseline variables were comparable to the baseline variables of the total cohort except for the following: median ESR of 33 (compared to 40 in the total cohort), median patient's global assessment of disease activity of 3.0 (compared to 4.9 in the total cohort), median Ritchie score of 8 (compared to 10 in the total cohort), 45% IgM RF positive (compared to 61.2% in the total cohort), 45% SE negative (compared to 31.9 % in the total cohort), and 25% presented with erosive disease with a median SH-score of 0 (compared to 37.5 % with erosive disease and a median SH-score of 1 in the total cohort). Thus, although these patients compared favorably with respect to the mentioned variables, they did not differ from the total cohort with respect to duration of symptoms and total swollen joint count, 2 variables that have been identified by our model as correlating strongly with disease outcome. However, since only a minority of all patients (12%) had a symptom duration < 6 weeks the contribution of this variable in the prediction model to identify individual patients is limited.

A possible explanation for difficulties in identifying mild RA in general could be that predictors of disease outcome in early RA (IgM RF positivity, swollen joint count, and disease duration) are sensitive but not specific for severe RA. As demonstrated by several prediction models developed in prospective observational studies, the percentage correctly classified with mild disease (specificity) is inversely related to the percentage of patients with severe disease: the higher the one, the lower the other (Table 6). In general, prediction of mild disease is more difficult than prediction of severe disease in the subgroup with early RA (Table 6).
The results of the hypothetical prediction model show that it is possible to identify patients with mild disease under the assumption that actual and predicted progression scores are normally distributed. This assumption is justified by the fact that residuals of

actual and predicted progression scores are both distributed approximately normally. In order to improve our results as well in the cohort as in the analytical calculations we performed the same analysis by using log-transformed actual and predicted progression scores, but this did improve neither the percentage of patients classified correctly or the comparability of results between the actual cohort and the analytical calculations.

Table 6. Prediction of radiological outcome in early RA: characteristics of prediction models in different studies

Study	No. of patients	Duration of follow-up, yrs	Definition of outcome	Proportion with severe outcome (%)	PPV† (%)	Sensitivity (%)	Specificity (%)
Van der Heijde (19)	147	2	Progression of joint damage ≥ 3	69	83	88	59
Van Zeben (21)	132 ♀	6 (mean)	Erosion score > 20	-	76‡	52	76
			> 60	-	78‡	88	48
Combe (24)	191	3	Progression yes/no	41	72	71	74
Dixey (25)	866	3	Erosive disease yes/no	58	68	78	52
			Severity of erosions	23	77	42	96

† PPV = Positive Predictive Value; ‡ proportion classified correctly (accuracy)

In the 95 patients selected for the regression analysis 69 patients (73%) turned out to have progression of radiographic damage, whereas 26 patients (27%) had no progression of radiographic damage during the first year. As the treatment of patients with RA has changed considerably over the last 2 decades the treatment prescribed to this group of patients is currently considered insufficient. Earlier and more aggressive intervention has been shown to result in lower joint damage progression rates (6,22). However, in the BeSt study, which compared aggressive and targeted treatment strategies, the proportion of patients with sequential monotherapy (starting with MTX) that showed progression of total SH score was 71%, a proportion very similar to the 73% in our study (23). In addition, given the high PPV of the described model, the number of patients incorrectly identified as having severe disease is low, independent of the proportion of patients with actual severe disease.

By using the developed prediction model, 44 patients (46.3 %) would have been classified as having high probability for severe (ie, progressive) disease. Only 3 patients (7%) were incorrectly identified as having severe disease. This latter proportion is surprisingly

low, since it was assumed that 80% certainty about the predicted disease outcome was required. The remainder of patients (53.7%) could not be classified as having either mild or severe disease.

Assuming that there are 2 possible treatment strategies for patients with early RA - (1) conventional treatment, for example DMARD monotherapy such as MTX, or (2) aggressive and eventually more expensive treatment, such as a combination of conventional DMARDs or the combination of MTX and TNF-α blocking drugs - these results lead to 3 different options in clinical practice.

The first option is to start with conventional therapy in all patients. Obviously this option rules out any possible benefit of early combination therapy. The second option is to start directly with combination therapy in all patients. In this case 69 patients with severe disease may be treated adequately, whereas all patients with mild disease will be overtreated and no patient will be undertreated. The last option is to start with combination therapy only in patients identified as having severe disease, while the remainder of patients will start with monotherapy. This way, 64 patients (67%) will receive adequate therapy (41 patients with severe disease and 23 patients with mild disease). In total, 28 of the patients with severe disease (41 %) will be undertreated. Of all patients receiving aggressive treatment, 7% (3 patients) will be overtreated. Overall, the risk of overtreatment varies from 7% to 27%, whereas the risk of undertreatment varies from 55% (28 out of 51) to 73%. Thus, by using a prediction model based on currently available predictive factors to differentiate treatment in individual patients the risk of overtreatment is 5-6 times less than the risk of undertreatment. Consequently, in the current situation a case can be made for aggressive intervention in all patients presenting with RA.

In summary, individualized treatment of early RA based on predicted disease outcome is currently possible in 46% of patients in our cohort. With better prediction models for disease outcome, individualized treatment of early RA becomes possible in up to 80% of patients, validating the search for better prognostic variables.

REFERENCES

1. van der Heide A, Jacobs JW, Bijlsma JW, Heurkens AH, Booma-Frankfort C, van der Veen MJ et al. The effectiveness of early treatment with "second-line" antirheumatic drugs. A randomized, controlled trial. Ann Intern Med 1996; 124(8):699-707.
2. van Jaarsveld CH, Jacobs JW, van der Veen MJ, Blaauw AA, Kruize AA, Hofman DM et al. Aggressive treatment in early rheumatoid arthritis: a randomised controlled trial. On behalf of the Rheumatic Research Foundation Utrecht, The Netherlands. Ann Rheum Dis 2000; 59(6):468-477.
3. Egsmose C, Lund B, Borg G, Pettersson H, Berg E, Brodin U et al. Patients with rheumatoid arthritis benefit from early 2nd line therapy: 5 year followup of a prospective double blind placebo controlled study. J Rheumatol 1995; 22(12):2208-2213.
4. Borg G, Allander E, Lund B, Berg E, Brodin U, Pettersson H et al. Auranofin improves outcome in early rheumatoid arthritis. Results from a 2-year, double blind placebo controlled study. J Rheumatol 1988; 15(12):1747-1754.
5. Lard LR, Visser H, Speyer I, vander Horst-Bruinsma IE, Zwinderman AH, Breedveld FC et al. Early versus delayed treatment in patients with recent-onset rheumatoid arthritis: comparison of two cohorts who received different treatment strategies. Am J Med 2001; 111(6):446-451.
6. Boers M, Verhoeven AC, Markusse HM, van de Laar MA, Westhovens R, van Denderen JC et al. Randomised comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis. Lancet 1997; 350(9074):309-318.
7. Mottonen T, Hannonen P, Leirisalo-Repo M, Nissila M, Kautiainen H, Korpela M et al. Comparison of combination therapy with single-drug therapy in early rheumatoid arthritis: a randomised trial. FIN-RACo trial group. Lancet 1999; 353(9164):1568-1573.
8. Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC et al. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. N Engl J Med 2000; 343(22):1586-1593.
9. Goekoop-Ruiterman YP, De Vries-Bouwstra JK, Van Zeben D, Kerstens PJ, Hazes JM, Zwinderman AH et al. Treatment strategies in early rheumatoid arthritis: clinical and radiological outcomes after 2 year follow-up of the BeSt study. Arthritis Rheum 2004; 50(12):4096.
10. Brennan P, Harrison B, Barrett E, Chakravarty K, Scott D, Silman A et al. A simple algorithm to predict the development of radiological erosions in patients with early rheumatoid arthritis: prospective cohort study. BMJ 1996; 313(7055):471-476.
11. Drossaers-Bakker KW, Zwinderman AH, Vlieland TP, van Zeben D, Vos K, Breedveld FC et al. Long-term outcome in rheumatoid arthritis: a simple algorithm of baseline parameters can predict radiographic damage, disability, and disease course at 12-year followup. Arthritis Rheum 2002; 47(4):383-390.
12. Scott DL. Prognostic factors in early rheumatoid arthritis. Rheumatology (Oxford) 2000; 39 Suppl 1:24-29.
13. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31(3):315-324.

14. Ritchie DM, Boyle JA, McInnes JM, Jasani MK, Dalakos TG, Grieveson P et al. Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. Q J Med 1968; 37(147):393-406.
15. van der Heijde DM, van Leeuwen MA, van Riel PL, Koster AM, 't Hof MA, van Rijswijk MH et al. Biannual radiographic assessments of hands and feet in a three-year prospective followup of patients with early rheumatoid arthritis. Arthritis Rheum 1992; 35(1):26-34.
16. van der Heijde DM, van Riel PL, Nuver-Zwart IH, Gribnau FW, vad de Putte LB. Effects of hydroxychloroquine and sulphasalazine on progression of joint damage in rheumatoid arthritis. Lancet 1989; 1(8646):1036-1038.
17. Bukhari M, Lunt M, Harrison BJ, Scott DG, Symmons DP, Silman AJ. Rheumatoid factor is the major predictor of increasing severity of radiographic erosions in rheumatoid arthritis: results from the Norfolk Arthritis Register Study, a large inception cohort. Arthritis Rheum 2002; 46(4):906-912.
18. Anderson JJ, Wells G, Verhoeven AC, Felson DT. Factors predicting response to treatment in rheumatoid arthritis: the importance of disease duration. Arthritis Rheum 2000; 43(1):22-29.
19. van der Heijde DM, van Riel PL, van Leeuwen MA, 't Hof MA, van Rijswijk MH, van de Putte LB. Prognostic factors for radiographic damage and physical disability in early rheumatoid arthritis. A prospective follow-up study of 147 patients. Br J Rheumatol 1992; 31(8):519-525.
20. Visser H, le Cessie S, Vos K, Breedveld FC, Hazes JM. How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis. Arthritis Rheum 2002; 46(2):357-365.
21. van Zeben D, Hazes JM, Zwinderman AH, Vandenbroucke JP, Breedveld FC. Factors predicting outcome of rheumatoid arthritis: results of a followup study. J Rheumatol 1993; 20(8):1288-1296.
22. Landewe RB, Boers M, Verhoeven AC, Westhovens R, van de Laar MA, Markusse HM et al. COBRA combination therapy in patients with early rheumatoid arthritis: long-term structural benefits of a brief intervention. Arthritis Rheum 2002; 46(2):347-356.
23. Goekoop-Ruiterman YP, De Vries-Bouwstra JK, Allaart CF, van Zeben D, Kerstens PJ, Hazes JM et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt Study). Arthritis Rheum. 2005; 52:3381-90.
24. Combe B, Dougados M, Goupille P, Cantagrel A, Eliaou JF, Sibilia J et al. Prognostic factors for radiographic damage in early rheumatoid arthritis: a multiparameter prospective study. Arthritis Rheum 2001; 44(8):1736-1743.
25. Dixey J, Solymossy C, Young A. Is it possible to predict radiological damage in early rheumatoid arthritis (RA)? A report on the occurrence, progression, and prognostic factors of radiological erosions over the first 3 years in 866 patients from the Early RA Study (ERAS). J Rheumatol Suppl 2004; 69:48-54.

Chapter 3

Biologics in early rheumatoid arthritis

J. K. de Vries-Bouwstra
B. A. C. Dijkmans
F. C. Breedveld

Rheum Dis Clin North Am 2005; 31 (4): 745-62.

ABSTRACT

Treatment of patients with rheumatoid arthritis (RA) with Disease Modifying Antirheumatic Drugs (DMARDs) is started immediately after diagnosis, resulting in more effective suppression of disease activity and substantial reduction of joint damage. The development of biologic agents has enabled remission as a realistic therapeutic goal in a greater proportion of patients. The tumor necrosis factor-α inhibitors, infliximab, etanercept, and adalimumab, have been studied in numerous randomized clinical trials. These agents can suppress disease activity directly, slow or stop progression of radiographic damage, and prevent further loss of quality of life. Patients treated with tumor necrosis factor-α inhibitors show few adverse events, which together with the high clinical effectiveness is favorable for treatment compliance. The exact role of these agents in the treatment of early-stage RA is unknown.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the synovial joints resulting in joint damage and loss of function. The consequences of RA can vary from hardly any impairment to severe disease with continuing high disease activity and progressive joint destruction resulting in severe functional decline and even increased mortality (1-3). The ultimate goal in managing RA is to prevent joint damage and to maintain functional ability. Evidence shows that substantial and irreversible joint damage already occurs within the first 2 years after disease onset (4). Current clinical practice is to treat earlier and more aggressive and is resulting in remarkably improved outcome of RA patients (5-8). Treatment with a combination of Disease Modifying Antirheumatic Drugs (DMARDs), with or without prednisone, early in the disease course has shown to be highly effective in slowing progression of joint damage (9-12). Intensive monitoring of disease activity and adjusting DMARD use accordingly have resulted in 65% of RA patients achieving remission (13). These findings are important because it has been shown that patients with persistent disease activity continue to develop joint damage and to lose functional capacity, which is directly related to increased morbidity and mortality (1-3).

Among the new therapeutic approaches of RA, the introduction of the biologic agents has established complete new standards for treatment of RA. The term *biologics* refers to a group of therapeutic agents that modify the biologic responses as observed in the pathophysiological inflammatory process in RA (14). Although traditional DMARDs generally slow joint damage progression, the prevention of joint damage has become a revolutionary possibility, particularly with the biologics inhibiting tumor necrosis factor (TNF)-α. Currently, infliximab, etanercept and adalimumab, all inhibiting TNF-α, are registered for treatment of RA. These TNF-α inhibitors suppress disease activity directly and powerfully and lower the disease burden significantly from the moment that treatment is started (15-17). Anakinra, a recombinant form of the naturally occurring interleukin-1 receptor antagonist, is also registered to treat RA. The clinical and radiographic effectiveness of anakinra is less convincing, however, compared with the effectiveness of the TNF-α inhibitors, and, to date, no trials with anakinra for early, DMARD-naive RA have been published (18). In contrast, numerous trials studying the effectiveness of infliximab, etanercept, and adalimumab in early RA have been performed.

It has been shown that a brief intervention early in the course of RA can "reset" radiographic progression rates during subsequent years independent of consequent therapy (11). In addition, delayed treatment trials have shown that a delay of only 3 to 9 months in starting DMARD therapy has a significant negative impact on radiographic outcome 2 years later (6,9). Both these observations support the existence of a "window of opportunity": a period in early-stage RA during which the progression rate of joint damage is set, and therapeutic interventions can exert maximum effects. Because the TNF-α inhibitors have been proved to stop joint damage progression in severe progressive RA, the achievements of these agents in early RA are currently of great interest. This article describes and discusses current knowledge for the use of TNF-α inhibitors in early RA.

INFLIXIMAB

Infliximab (cA2 MAb, Remicade) is a chimeric human/ mouse monoclonal antibody against TNF-α consisting of human IGg1 and murine Fv that binds with high affinity and specificity to human, soluble and membrane bound TNF-α (14,18). Infliximab is administered intravenously, and the distribution is mainly intravascular with half-life of 8-12 days. Treatment of RA with infliximab has been developed and approved in combination with methotrexate.

The effectiveness of infliximab has been proved by results of clinical trials evaluating the additional effect of infliximab to methotrexate compared with placebo in active long-standing RA (19,20). The Anti-TNF Trial in Rheumatoid Arthritis with Concomitant Therapy (ATTRACT) (16,20,21) showed that the combination of infliximab and methotrexate was superior to methotrexate alone for clinical and radiographic outcome at weeks 30 and 54. This study included 428 patients with active RA despite treatment with methotrexate in a minimum dose of 12.5 mg/wk, who were allocated randomly to receive placebo or one of four regimens of infliximab: 3 mg/kg or 10 mg/kg, every 4 or every 8 weeks. For the subgroup of 82 patients with early RA (disease duration ≤ 3 years), these results were comparable. A further subset analysis of these 82 patients with early RA was performed to evaluate radiographic damage at week 102 (22). Compared with all ATTRACT patients, these early RA patients had lower baseline Sharp-van der Heijde-scores (SHS) (median total score 18.0 compared with 51.5), but higher predicted annual progression rate (median 11.3 compared with 7.3). Radiographs were available for 61 of 82 patients: 12 patients receiving methotrexate and placebo and 49 patients receiving methotrexate and infliximab. Early RA patients receiving methotrexate alone showed significant progression of radiographic damage (median total ΔSHS 14.3), whereas progression of joint damage was almost totally inhibited in early RA patients receiving the combination of methotrexate and infliximab (median total ΔSHS 0.5). The progression rate in the early RA group receiving methotrexate alone was higher compared with the progression rate in all patients in the ATTRACT study receiving methotrexate alone. In the early RA patients receiving methotrexate and infliximab, the progression of radiographic damage was comparable to all patients receiving the combination, with the lowest infliximab dose regimen being equally effective as the higher doses of infliximab.

Given the small sample size and the nature of the analysis, conclusions based on this study must be limited. Nevertheless, the results of this subanalysis indicate that the additional effect of infliximab is even greater in patients with early RA compared with the effectiveness in long-standing disease.

Placebo-controlled studies of infliximab in early rheumatoid arthritis

In November 2004, the results of a double-blind, placebo-controlled trial comparing the combination infliximab and methotrexate with methotrexate alone in early RA (≤ 3 years) were published (23). A total of 1049 methotrexate-naive patients with active disease

(≥ 10 swollen joints, ≥ 12 tender joints and at least 1 of the following: rheumatoid factor positivity, radiographic erosions of the hands or feet, or C-reactive protein [CRP] ≥ 2 mg/dL) were included. At baseline, mean disease duration was 0.9 year, and more than 80% of patients had erosive disease. All patients received methotrexate, 20 mg/ wk, combined with either placebo or infliximab, 3 mg/kg, or infliximab, 6 mg/kg, infusions at 0, 2, and 6 weeks and thereafter every 8 weeks until week 46. After 54 weeks of follow-up, patients treated with the combination methotrexate and 3 mg/kg or 6 mg/kg of infliximab showed a higher percentage of overall American College of Rheumatology (ACR) improvement from baseline compared with patients receiving placebo (median 38.9% and 46.7% compared with 26.4%; $P < 0.001$). Among patients receiving infliximab the percentages fulfilling ACR20, ACR50, ACR70, and ACR90 were significantly higher compared with placebo. For placebo, ACR20 was 53.6%, ACR50 was 32.1%, ACR70 was 21.2%, and ACR90 was 6.6%. For infliximab, 3 mg/kg, ACR20 was 62.4%, ACR50 was 45.6%, ACR70 was 32.5%, and ACR90 was 10.0%, for infliximab, 6 mg/kg, ACR20 was 66.2%, ACR50 was 50.4%, ACR70 was 37.2%, and ACR90 was 16.9% (Table 1). The percentage of patients achieving remission ($DAS_{28} < 2.6$) was significantly higher in the patients receiving infliximab: 15% for placebo compared with 21% for infliximab, 3 mg/kg, and 31% for infliximab, 6 mg/kg. Mean total progression of radiographic joint damage (SHS) was 3.7 for the placebo-group, 0.4 for the infliximab, 3 mg/kg, group and 0.5 for the infliximab, 6 mg/kg, group ($P < 0.001$). In the placebo group, 11% of patients (n = 31) showed progression greater than the smallest detectable difference ($\Delta SHS > 9.03$); in the infliximab, 3 mg/kg, group, 3.9% (n = 14), and in the infliximab, 6 mg/kg, group, 1.9% (n = 7); $P < 0.001$ for placebo compared with either infliximab group). Compared with patients receiving methotrexate alone, the combination with infliximab resulted in an additional 11% of patients improving at least 0.22 points in the Health Assessment Questionnaire (HAQ) score, which is set as a clinically relevant improvement. The proportion of patients with one or more serious adverse events was higher in the methotrexate and infliximab group (14%) than in the methotrexate and placebo group (11%) with significantly more serious infections (2.1% versus 5.6% and 5.0% for placebo, infliximab, 3 mg/kg, and infliximab, 6 mg/kg). In particular, community-acquired pneumonia occurred more frequently in the patients receiving infliximab, with an observed incidence of 2% compared to 0% for placebo.

The results of this study show the additional clinical, functional and radiographic benefits of methotrexate and infliximab compared with methotrexate alone in patients with early RA with a poor prognosis. Beneficial effects of methotrexate monotherapy also were underlined with 65% of these patients showing a clinically relevant improvement of functional ability (HAQ score) and with a median progression of erosions of 0.3 point. Given the study population with highly active RA, the higher percentage of serious infections in the infliximab group, and the observed benefits of methotrexate monotherapy, this study does not absolutely prove the superiority of the combination with infliximab for all individual patients with early RA.

To investigate the possibility of monitoring of treatment success in terms of joint damage, Taylor and colleagues (24) performed a placebo-controlled, randomized clinical

trial in 24 patients with early RA with ultrasound assessments at week 18 to predict the development of joint damage after 1 year of treatment with infliximab. Eligible patients had symptoms for a maximum of 3 years, were IgM rheumatoid factor positive, had at least one erosion in a metacarpophalangeal joint, and were treated with oral methotrexate for at least 8 weeks, in a stable dose (12.5 – 17.5 mg/wk). All patients received infusions at weeks 0, 2, and 6, and then every 8 weeks until week 46, 12 patients with infliximab, 5 mg/kg, and 12 patients with placebo. Methotrexate was continued at baseline dose until week 18. Thereafter, methotrexate was increased to a maximum of 25 mg/wk if a 50% reduction in number of swollen joints in the hands and wrists was not achieved. The mean disease duration of included patients was 1.5 years, with a mean duration of treatment with methotrexate (mean dose 15 mg/wk) of 11 months. At week 18, there was a significant higher reduction of synovial thickness in the infliximab group (-50% versus +1.2%; $P = 0.014$). After 54 weeks, progression of total SHS was greater in the patients receiving placebo compared with the patients receiving infliximab (median 14.0 [interquartile range 17] versus median 3.3 [interquartile range 3.3]; $P = 0.056$). At week 54, patients in the infliximab group showed a moderate or good clinical response (DAS_{28} and ACR response levels) more often, without the need to increase methotrexate dosage (median total increase of methotrexate 0 mg/wk compared with 6.3 mg/wk in the placebo group; $P = 0.001$). Remarkably, correlations of baseline synovial thickness and baseline synovial vascularity with progression of SHS were strongly positive in the placebo group, but weakly negative and nonsignificant in the infliximab group.

These results suggest that patients with early RA and high baseline disease activity may benefit most from treatment with infliximab with respect to progression of radiographic damage, and that indicators for poor prognosis at baseline are overruled by treatment with infliximab.

Infliximab as remission-induction therapy

Some small studies have been performed to test the hypothesis whether early treatment with TNF-α inhibitors in early RA patients with poor prognosis results in sustained improvement of outcome. Conaghan and colleagues (25) conducted a pilot study in five patients with newly diagnosed RA to investigate whether high-dose induction therapy with infliximab results in long-term, drug-free remission. Included patients had a mean symptom duration of 7.3 months, were DMARD-naive and fulfilled three or more criteria for poor prognosis. Patients were treated with methotrexate, 15 mg/wk, combined with infliximab, 10 mg/kg, at weeks 0, 2, 6, and 10 with an optional extra infusion at week 12 if remission was not achieved. After the initial four infusions, remission was achieved in one patient, three patients showed highly significant improvement (two ACR70 response and one ACR50 response), and one patient showed no improvement. One additional infusion did not result in further clinical improvement, and drug-free remission was achieved in none of the patients.

A double-blind, placebo-controlled study was performed in 20 DMARD-naive patients with early RA with poor prognosis (mean symptom duration of 6 months) (26).

Table 1. Efficacy of methotrexate, TNF-α inhibitors and the combination TNF-α inhibitor and methotrexate in early rheumatoid arthritis

Trial				Population		Clinical Outcome (% of patients)				Radiographic Outcome
Name [Ref]	Type	Comparison	Follow-up (wk)	No. of patients	Disease duration	Regimen	ACR50	ACR70	Remission (DAS_{28}<2.6)	Δ SHS[a] (mean)
Aspire [23]	RCT	MTX alone vs. MTX + infliximab	54	1049	≥3 mo and ≤3 y	MTX + placebo MTX + IFX 3 mg/kg MTX + IFX 6 mg/kg	32 46* 50*	21 33** 37*	15 21*** 31*	3.7 0.4* 0.5*
TEMPO [30]	Subanalysis of RCT	MTX + etanercept vs. either drug alone	104	229 (of 682)	≥6 mo and ≤3 y	MTX + placebo Etanercept + placebo MTX + etanercept	43 58 69****	23 33 44****	19 34 43	- - -
PREMIER [39]	RCT	MTX + adalimumab vs. either drug alone	104	799	<3 y	MTX Adalimumab MTX + adalimumab	43 37 59*****	28 27 47*****	25 25 50*****	10.4 5.5****** 1.9*****

Abbreviations: MTX = methotrexate; RCT = randomized controlled trial. [a] Progression of joint damage as measured with Sharp-van der Heijde score.
* $P < 0.001$ compared to MTX + placebo; ** $P = 0.002$ compared to MTX + placebo; *** $P = 0.065$ compared to MTX + placebo;
**** $P < 0.05$ compared to MTX alone; *****$P < 0.001$ compared to MTX alone and to adalimumab alone; ****** $P < 0.001$ compared to MTX alone.

All patients were treated with methotrexate combined with either placebo or infliximab, 3 mg/kg, during 1 year, and patients were followed in an open-label fashion for another year. Patients receiving infliximab showed significantly greater reduction of synovitis as measured by MRI of the second through fifth metacarpophalangeal joints (t = 4, 14 and 54 weeks), a lower erosion score (t = 54 weeks), and better functional improvement (HAQ; t = 54 weeks). After a mean of 90 weeks of follow-up (ie, after a mean of 44 weeks without infliximab), none of the patients fulfilling ACR50 response at t = 54 week (78%) had experienced a flare of disease activity (median $DAS_{28} < 2.6$). This study indicates that by starting treatment with a combination of methotrexate and infliximab, sustained clinical improvement can be achieved even after stopping infliximab. That these results are not fully confirmed by the study of Conaghan and colleagues (25) can be explained partly by the differences in treatment period and outcome measures (drug-free remission versus ACR50 response).

ETANERCEPT

Etanercept (TNFR:Fc, Enbrel) is a dimeric recombinant fusion protein consisting of the extracellular portion of the human p75-TNF receptor type II and the Fc portion of a type 1 human immunoglobulin (IgG1). Etanercept acts as a competitive inhibitor of TNF by binding primarily soluble TNF-α and TNF-β. Etanercept has a half-life of ± 3 days and is administered subcutaneously twice weekly. It is registered as monotherapy for RA (14,18).

The clinical efficacy and safety of etanercept were shown in several randomized, double-blind, and placebo-controlled trials in patients with active RA despite DMARD treatment (27,28). These trials generally showed that etanercept is well tolerated and results in rapid and sustained clinical and functional improvement, also when added to methotrexate. A head-to-head comparison of the combination etanercept and methotrexate with either drug alone was performed, the Trial of Etanercept and Methotrexate with Radiographic Patient Outcomes (TEMPO)(17). This double-blind, placebo-controlled trial comprising 682 patients with active RA and disease duration between 6 months and 20 years (mean 6.6 years) showed that the combination was superior to either drug alone. Included patients had previously failed on at least one DMARD other than methotrexate and received either etanercept, 25 mg twice weekly; oral methotrexate, 7.5 mg/wk to 20 mg/wk; or the combination. After one year, the percentage of patients achieving ACR50 and ACR70 responses was consistently and significantly higher in the combination group compared with each other group. At week 52, 35% of the patients receiving the combination achieved clinical remission (DAS < 1.6) compared with 13% of the patients on methotrexate and 16% of the patients on etanercept. The progression of joint damage over 52 weeks was lowest in the combination group, with a significant lowering of total SHS compared with baseline. Evaluation of the progression of radiographic damage over 2 years in 622 patients with at least one follow-up x-ray showed lack of progression of total SHS ($\Delta SHS \leq 0.5$) in 60%, 68%, and 78% of patients in the methotrexate, etanercept,

and combination groups. The combination therapy resulted in sustained and significant lowering of total SHS compared with baseline, suggesting that repair of damage is possible with the combination methotrexate and etanercept (29).

Of all patients participating in the TEMPO trial, approximately 30% had at baseline a disease duration of less than 3 years. After 2 years of follow-up, a post hoc analysis was performed to evaluate the therapeutic effect of the combination of etanercept and methotrexate (n = 77) compared with either drug alone (n = 77 for etanercept and n = 75 for methotrexate) in patients with early-stage RA (30). Patients receiving the combination showed significantly higher clinical response and better functional improvement compared with patients receiving either methotrexate or etanercept. The proportion of patients achieving ACR50 was 43%, 58%, and 69%, and the proportion of patients achieving ACR70 was 23%, 33%, and 44% for methotrexate, etanercept, and the combination (Table 1). Remission (DAS < 1.6) was achieved in 19% of the patients on methotrexate, in 34% of the patients on etanercept and in 43% of the patients on the combination. Overall, therapeutic responses of early RA patients were comparable to the responses of all patients participating in the TEMPO trial.

Altogether these results strongly suggest that the combination of methotrexate and etanercept is superior to etanercept monotherapy, especially with respect to the measurements indicating excellent clinical responses (ACR50, ACR70, DAS remission, and complete absence of radiographic progression). The subanalysis in early RA patients did not show additional benefits of the combination for this group of patients.

Randomized controlled trial with etanercept in early rheumatoid arthritis

The Early Rheumatoid Arthritis (ERA) trial is the first clinical trial designed and performed to compare biologic monotherapy with a nonbiologic DMARD for the treatment of early RA patients with poor prognosis (31). In this randomized controlled trial, 632 patients with early RA were followed for at least 1 year in a double-blind fashion. The patients included in this study had a mean disease duration of 12 months; had active RA with 10 or more swollen joints, 12 or more tender joints, an erythrocyte sedimentation rate greater than or equal to 28 mm, CRP greater than or equal to 2 mg/dL, or morning stiffness lasting 45 minutes or longer; and were at high risk for radiographic progression with positive rheumatoid factor or at least three erosions in hands or feet. Prior treatment with methotrexate was not allowed. Patients were randomized to receive either twice-weekly etanercept (10 mg or 25 mg) or weekly oral methotrexate (mean dose 19 mg/wk). After the blinded phase of the trial, 512 patients continued to receive the allocated treatment for an additional year in an open-label extension study (32).

Patients receiving etanercept showed more rapid clinical improvement compared with patients receiving methotrexate. During the 12 months of follow-up, patients receiving etanercept, 25 mg, had significantly better overall clinical response (area under the curve for ACR-N) compared with patients on methotrexate. The percentages of patients fulfilling ACR20, ACR50, and ACR70 were significantly different between the methotrexate group and

the etanercept, 25 mg, group during the first 6 months and comparable for all groups thereafter, although etanercept, 25 mg, was numerically superior to methotrexate. At 2 years of follow-up, the ACR20 response was significantly higher in the etanercept, 25 mg, group than in the methotrexate group (72% versus 59%; $P = 0.005$). Progression of radiographic damage was significantly lower in the etanercept, 25 mg, group compared with the methotrexate group and the etanercept, 10 mg, group. Of patients receiving methotrexate, 51% had no increase in total Sharp-van der Heijde score ($\Delta SHS < 0.5$) compared with 63% of patients receiving 25 mg etanercept ($P = 0.017$), and 58% compared with 70% had no increase in the erosion score ($P = 0.012$). After 1 year of follow-up, 55% of patients receiving methotrexate and patients receiving etanercept, 25 mg, showed a clinically relevant improvement in functional ability (ΔHAQ from baseline > 0.5). At 2 years of follow-up, this percentage remained stable for the etanercept, 25 mg, group, whereas the percentage in the methotrexate group declined to 37% ($P < 0.001$). During the 2-year period, significantly more patients receiving methotrexate discontinued therapy because of adverse events (12% for methotrexate and 5% and 7% for etanercept, 10 mg and 25 mg).

After the follow-up phase of 2 years, patients were followed in an open-label fashion. Patients on etanercept, 10 mg or 25 mg, all continued with etanercept, 25 mg. In patients receiving methotrexate, etanercept, 25 mg, was added to methotrexate, or methotrexate was replaced by etanercept, 25 mg. At 3 years of follow-up, the patients who switched from methotrexate or etanercept, 10 mg, showed additional benefit of etanercept, 25 mg, with approximately equal percentages of patients fulfilling ACR20 as in the patients who initially were allocated to etanercept, 25 mg, (ACR20 72%, 81%, and 76%). Observations at 3 and 4 years of follow-up showed sustained safety and clinically efficacy of treatment with etanercept, 25 mg (33,34).

The results of the ERA trial show that over 2 years of follow-up, etanercept, 25 mg, is superior to methotrexate in reduction of progression of joint damage and maintaining functional improvement in early, aggressive RA. This trial and the TEMPO trial underline the benefits of methotrexate as monotherapy, however, resulting in arrest of progression of joint damage in 51% (ERA) and 60% (TEMPO) of the patients. In the ERA trial, clinical improvement is more rapid with etanercept, but is comparable with methotrexate after the initial 6 months, and by switching to etanercept, 25 mg, after 2 years, equal clinical improvement can be achieved. Additional analyses need to determine whether the initial rapid clinical improvement results in ongoing lower progression of joint damage and sustained better physical function. Because an improvement in total SHS was observed in the patients on the combination in the TEMPO trial, the course of radiographic damage during long-term follow-up of the ERA-patients would be interesting.

Etanercept in early rheumatoid arthritis: relative benefits compared with established rheumatoid arthritis

That the initial phase may prove to be crucial in future functional ability is indicated by the results of the post-hoc analysis comparing functional improvement of early RA patients with that of patients with established RA, both receiving etanercept (35). The early RA

patients comprised the 207 patients participating in the ERA trial and randomized to receive etanercept, 25 mg, twice weekly. For the established RA group, 464 patients were derived from a cohort in which long-term safety of etanercept monotherapy was studied (36). Before start of etanercept, this latter group of patients had had suboptimal responses with one or more DMARDs, including methotrexate. These patients were treated with either 10 mg or 25 mg of etanercept twice weekly, with 84% of all patients receiving 25 mg during the whole study period. At baseline, the patients with established RA had a higher mean disease duration of 12 years compared with 1 year for the ERA group, higher mean HAQ scores (1.64 versus 1.45; $P = 0.004$), and higher mean CRP levels (4.5 versus 3.3; $P = 0.005$), but were comparable with the ERA group for other baseline characteristics. HAQ scores during 3 years of follow-up were compared between the two groups with HAQ scores available for 3 years (148 ERA patients [71%], 288 established RA patients [62%]). In both groups, rapid, sustained clinical improvement was achieved with etanercept with percentages of patients achieving ACR20, ACR50, and ACR70 responses at 3 years of 76%, 56% and 33% in the ERA group compared with 77%, 50% and 28% in the established RA group. Improvement of function also was rapid and sustained in both groups with more than 50% of patients already improved more than 0.2 points in HAQ (standing for clinically relevant improvement) at 2 weeks after start of etanercept. The magnitude of improvement was significantly greater, however, in patients with early RA. At 3 years of follow-up, more early RA patients had achieved clinically relevant functional improvement (85%) compared with patients with established RA (75%; $P = 0.0125$ corrected for baseline HAQ). An explanation for the observed difference in improvement of function may be the underlying existing joint damage in the patients with established RA, whereas in the ERA patients, joint inflammation is the main cause of functional impairment.

Although the analysis has obvious limitations - a post-hoc analysis on two populations from different studies with the patients with established RA already having failed on methotrexate - this study emphasizes that early intervention results in better functional improvement. Long-term follow-up of the progression of radiographic joint damage in both of these groups and in the different groups originally started in the ERA trial needs to clarify whether the initial rapid clinical improvement will turn out to be crucial in terms of future joint damage progression and further functional decline.

ADALIMUMAB

Adalimumab (D2E7, Humira) is a fully human monoclonal TNF-α antibody with high specifity and affinity for TNF-α. It is structurally and functionally analogous to naturally occurring human IgG1 with a half-life of approximately 14 days, and it exerts its therapeutic effects by blocking the interaction between TNF-α and the TNF cell surface receptors. Adalimumab was developed for treatment of RA as monotherapy and in combination with methotrexate and is administrated by subcutaneous injections. Currently, adalimumab is approved for treatment of RA as monotherapy (14,18).

The ARMADA trial (Anti-TNF Research Study Program of the Monoclonal Antibody Adalimumab in RA) evaluated the efficacy and safety of adalimumab in combination with methotrexate in patients with active RA despite methotrexate and with mean disease duration of 12.3 years (37). All groups receiving methotrexate and adalimumab showed significant higher clinical response rates; adalimumab was well tolerated.

Radiographic benefits of adalimumab added to continuing methotrexate therapy were investigated in a placebo-controlled study with 619 patients with mean disease duration of 10.9 years and previous incomplete responses to methotrexate (15). The progression of radiographic joint damage was significantly lower in patients receiving adalimumab at 24 and 52 weeks. After one year, 62% and 58% of patients on adalimumab (40 mg every other week and 20 mg/wk) compared with 46% of patients taking placebo had not developed new erosions.

The clinical efficacy and safety of adalimumab as monotherapy were evaluated in a 26-week, double-blind, placebo-controlled trial with 544 patients with long-standing, severe RA despite treatment with at least one DMARD and mean disease duration of 11 years (38). Treatment with adalimumab as monotherapy resulted in rapid and sustained clinical improvement, with more patients achieving ACR20, ACR50, and ACR70 compared with placebo. Patients on adalimumab showed better functional improvement. The number of adverse events per total years of treatment was comparable between adalimumab and placebo, and serious infections were reported equally in both groups.

Adalimumab in early rheumatoid arthritis

The following results were published concerning the effectiveness and safety of adalimumab in early RA. The PREMIER study compared the combination of methotrexate (rapidly increased to 20 mg/wk) and adalimumab (40 mg every other week) to either drug alone over two years (39). A total of 799 methotrexate-naive patients with active RA of less than 3 years' duration (mean duration of 0.7 year) were included. Patients receiving the combination showed rapid, sustained, clinical improvement with greater proportions of patients fulfilling ACR20, ACR50, and ACR70 starting from week 2 during the entire 2-year study period, with ACR50 response at 1 year of 61%, 42%, and 46% and at 2 years of 59%, 37%, and 43%, and with ACR70 response at 1 year of 46%, 26%, and 28% and at 2 years of 47%, 27%, and 28% for the combination, adalimumab and methotrexate (Table 1). Of patients receiving the combination, 49% achieved a continuous ACR70 response for 6 months or more compared with 25% and 27% for adalimumab and methotrexate alone. More patients receiving the combination achieved remission ($DAS_{28} < 2.6$): 50% for the combination compared with 25% for both monotherapy groups at 2 years of follow-up. Compared with patients receiving methotrexate monotherapy, patients receiving adalimumab had lower progression of total SHS with mean change of SHS over 2 years of 1.9, 5.5, and 10.4 for the combination, adalimumab alone, and methotrexate alone. There were no differences in frequencies of adverse among the three groups. This study shows the clinical and radiographic benefits

of adalimumab and methotrexate in early RA compared with each drug alone with a remarkably high percentage of patients on the combination achieving remission (50%) and sustained ACR70 (49%).

An open-label extension study evaluated the prevalence, sustainability, and clinical features of persistent remission in 846 patients with early or long-standing RA all treated with adalimumab 40 mg every other week, and methotrexate (40). The period in persistent remission was calculated from the first to the last visit continuously showing a DAS_{28} less than 2.6 for 6 months or more with one DAS_{28} 2.6/year or greater allowed. A total of 29% of patients achieved clinical remission after a mean period of 10 months, and remission was sustained for a mean period of 25 months (range 6-56). Of patients with early RA (≤ 2 years) 31% achieved remission compared with 29% of patients with longstanding disease. Mean time to remission and mean duration of remission were comparable between patients with early and patients with longstanding RA. The 36 patients with early RA who achieved remission had a higher CRP and DAS_{28} and longer duration of morning stiffness at baseline compared with the 209 patients with more long-standing RA (> 2 years) who achieved remission. This finding suggests that the combination of methotrexate and adalimumab can have even more impact on disease course when applied early.

SAFETY CONSIDERATIONS

Accompanying the great successes of the TNF-α inhibitors, there have been concerns about the safety profile. Because TNF-α is a key proinflammatory cytokine playing an important role in the host defense-system, it was reasoned that TNF-α blockade could lead to increased incidence of infections and malignancies in particular. Present-day knowledge is based mainly on long-term follow-up of clinical trials, post marketing surveillance, and case reports and notably concerns patients with long-standing and refractory RA. Several issues have emerged from this follow-up, including injection site and infusion reactions, serious infections including tuberculosis, and lymphomas. Injection site reactions and infusion reactions are mostly mild to moderate, are manageable, and rarely lead to discontinuation of therapy. Special consideration has to be made in the occurrence of problems including serious infections and tuberculosis. In the latter, a detailed evaluation for latent tuberculosis should be made before starting therapy. Careful monitoring is necessary to minimize the risk for other opportunistic and serious infections. In case of an infection, standard medical treatment generally is sufficient. TNF-α inhibitors should not be started or should be discontinued until the infection has been treated adequately. Data about the risk of developing lymphomas are difficult to interpret because the incidence of lymphomas is increased in RA (41). Treatment with TNF-α inhibitors is associated with increased standardized incidence ratios for lymphomas, but the differences between various treatment groups are very little. Part of the difference may be due to the application of TNF-α inhibitors preferentially in patients with long-standing and severe RA who might have a higher risk to develop lymphomas in general.

Other issues related to the TNF-α inhibitors, such as autoimmune disorders, cardiac insufficiency, demyelinating diseases, and interstitial lung disease also may need to be considered even tough data for these are weak. If the patients are informed about the various safety aspects and outcomes, the risk-to-benefit ratio for treatment with TNF-α inhibitors remains favorable. Long-term follow-up of various groups of RA patients needs to elucidate whether the risk-to-benefit ratio of patients with early RA is comparable to patients with established disease (41-43).

THERAPEUTIC STRATEGY IN EARLY RHEUMATOID ARTHRITIS

The Aspire study, the TEMPO trial, and the PREMIER study all independently showed the superiority of the combination of methotrexate and biologic in early and in long-standing RA. Each of these studies also emphasized the effectiveness of methotrexate and biologic monotherapy, however, and left unanswered which treatment strategy should be employed in patients presenting with RA. Should therapy be started with one DMARD, which, if unsuccessful, would be replaced with another DMARD or with a TNF-α inhibitor? Or should treatment be aggressive from the beginning with a combination of proven DMARDs or with a combination including a TNF-α inhibitor?

To find out which strategy would yield the best outcome, a head-to-head comparison of four aggressive treatment strategies in patients with early, DMARD-naive RA was made, the BeSt study (44). This randomized, single-blinded clinical trial compared sequential monotherapy, step-up combination therapy, initial combination therapy, and initial biologic therapy for functional ability and radiographic damage. The common goal in all strategies was to reduce disease activity rapidly and persistently by tight monitoring and immediate adjustment of therapy in case of an insufficient response (DAS > 2.4). If the clinical response was consistently adequate (DAS ≤ 2.4 for at least 6 months), medication was tapered until only one drug remained. A total of 508 patients were included with median symptom duration of 23 weeks and with active RA (≥ 6 swollen and ≥ 6 tender joints, erythrocyte sedimentation rate ≥ 28 mm/hr or visual analog scale global health ≥ 20 mm). After 2 years, 42% of patients in all groups were in clinical remission (DAS < 1.6), showing that very low disease activity is an achievable goal with aggressive strategies. Over 2 years of follow-up, cumulative disease activity and cumulative functional impairment were lower in the initial combination group and the initial biologic group. These latter groups especially showed a more rapid improvement of DAS and HAQ during the first 3 months. At 1 and 2 years, mean DAS was equal for all four groups, and mean HAQ was only significantly higher in the sequential monotherapy group compared with the initial combination and the initial biologic groups. Progression of radiographic joint damage was significantly lower for the groups starting with a combination or a biologic, although the differences were small with median ΔSHS of 2.0 for sequential monotherapy and step-up and 1.0 for the groups with initial combination or a biologic. The number of adverse events was equal for all groups.

Data for the number of treatment switches varied among the four groups with in general fewer adjustments in the groups starting with a combination or a biologic. In the group with sequential monotherapy, in which in sequential order therapy could be switched from methotrexate to sulphasalazine to leflunomide to methotrexate plus infliximab, 33% of patients still received methotrexate at the end of the second year. Consequently, in approximately one third of patients with early RA, a low disease activity could be maintained with methotrexate monotherapy. In the group with the biologic, patients started with methotrexate, 25 mg/wk, plus infliximab, 3 mg/kg at 0, 2, and 6 weeks and every 8 weeks thereafter. Disease activity was assessed before each infliximab infusion. If the DAS was greater than 2.4, the infliximab dose was increased to 6, 7.5, and 10 mg/kg, and finally patients were switched to sulphasalazine. In case of consistently adequate response, infliximab was tapered and finally discontinued, and next methotrexate was tapered to 10 mg/wk. Of all patients who started with methotrexate and infliximab, 72% remained in the initial treatment step. During the 2-year study period, infliximab could be discontinued after an average of 1 year without disease flare in 54% of the patients, and methotrexate afterward could be tapered to a mean dose of 12.2 mg/wk. SHS progression was observed less frequently in responders compared with patients who failed on treatment with increasing dosages of infliximab. In summary, after 2 years of follow-up, 33% of patients in group 1 compared with 54% of patients in group 4 were receiving methotrexate monotherapy.

All patients participating in the BeSt study were asked whether their general health was improved with treatment. Overall the results were positive with optimal results in the biologic group, in which 91% of patients stated that their general health had improved by therapy. This group was followed in descending order by sequential monotherapy (87%), step-up (85%), and combination therapy (77%).

SUMMARY

Trials as TEMPO, Aspire, BeSt, and PREMIER all show that treatment with a TNF-α inhibitor, infliximab, etanercept, or adalimumab, especially in combination with methotrexate is a step forward in treatment of early RA. Long-term follow-up of patients treated early with a biologic has to clarify certain issues, however, as listed in Box 1:

Box 1. Issues in long-term follow-up of patients treated early with biologics

- Timing: Whether the impact of the combination biologic and methotrexate applied during the early stages of RA (window of opportunity) will turn out to be crucial for long-term joint damage progression needs to be clarified.
- Costs: Because biologics currently are far more expensive than the traditional DMARDs, data concerning absenteeism and additional use of health-care services are important.
- Disease severity: Biologics have proven to work well in severe, active RA, but might work even better in moderate disease.
- Safety: Current knowledge is based mainly on long-term follow-up in patients with long-standing RA; long-term follow-up of early RA patients needs to clarify the risk-profile for these patients.
- Other biologics: Apart from the TNF-α inhibitors, anakinra and a variety of other agents have been developed and are currently under investigation.

Additional observations and long-term follow-up on the use of biologics in various groups of patients need to point out the exact balance in terms of cost and benefit for the individual patient with early RA. Overall, the promising results of the trials discussed in this article indicate that the treatment paradigm for RA has been shifted toward using TNF-α inhibitors. By defining high treatment goals and by the use of aggressive treatment strategies, the best outcome is obtained, and remission or low disease activity becomes an achievable goal for a greater proportion of patients. Success or failure of treatment should be evaluated regularly as part of every day practice. Because current data show that TNF-α plays a key role in disease progression, TNF-α inhibitors should be considered for use as soon as traditional therapies do not result in the desired treatment goal. When severe joint damage has developed, TNF-α inhibitors can improve disease status significantly. If there is no severe, irreversible destruction, the use of TNF-α inhibitors would have a greater impact on functional ability by preservation of the joints.

REFERENCES

1. Pincus T, Callahan LF, Sale WG, Brooks AL, Payne LE, Vaughn WK. Severe functional declines, work disability, and increased mortality in seventy-five rheumatoid arthritis patients studied over nine years. Arthritis Rheum 1984; 27(8):864-872.
2. Wolfe F, Mitchell DM, Sibley JT, Fries JF, Bloch DA, Williams CA et al. The mortality of rheumatoid arthritis. Arthritis Rheum 1994; 37(4):481-494.
3. Wolfe F. The natural history of rheumatoid arthritis. J Rheumatol Suppl 1996; 44:13-22.
4. Mottonen TT. Prediction of erosiveness and rate of development of new erosions in early rheumatoid arthritis. Ann Rheum Dis 1988; 47(8):648-653.
5. Boers M, Verhoeven AC, Markusse HM, van de Laar MA, Westhovens R, van Denderen JC et al. Randomised comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis. Lancet 1997; 350(9074):309-318.
6. Lard LR, Visser H, Speyer I, vander Horst-Bruinsma IE, Zwinderman AH, Breedveld FC et al. Early versus delayed treatment in patients with recent-onset rheumatoid arthritis: comparison of two cohorts who received different treatment strategies. Am J Med 2001; 111(6):446-451.
7. van Jaarsveld CH, Jacobs JW, van der Veen MJ, Blaauw AA, Kruize AA, Hofman DM et al. Aggressive treatment in early rheumatoid arthritis: a randomised controlled trial. On behalf of the Rheumatic Research Foundation Utrecht, The Netherlands. Ann Rheum Dis 2000; 59(6):468-477.
8. van der Heide A, Jacobs JW, Bijlsma JW, Heurkens AH, Booma-Frankfort C, van der Veen MJ et al. The effectiveness of early treatment with "second-line" antirheumatic drugs. A randomized, controlled trial. Ann Intern Med 1996; 124(8):699-707.
9. Egsmose C, Lund B, Borg G, Pettersson H, Berg E, Brodin U et al. Patients with rheumatoid arthritis benefit from early 2nd line therapy: 5 year followup of a prospective double blind placebo controlled study. J Rheumatol 1995; 22(12):2208-2213.
10. Korpela M, Laasonen L, Hannonen P, Kautiainen H, Leirisalo-Repo M, Hakala M et al. Retardation of joint damage in patients with early rheumatoid arthritis by initial aggressive treatment with disease-modifying antirheumatic drugs: five-year experience from the FIN-RACo study. Arthritis Rheum 2004; 50(7):2072-2081.
11. Landewe RB, Boers M, Verhoeven AC, Westhovens R, van de Laar MA, Markusse HM et al. COBRA combination therapy in patients with early rheumatoid arthritis: long-term structural benefits of a brief intervention. Arthritis Rheum 2002; 46(2):347-356.
12. Verstappen SM, Jacobs JW, Bijlsma JW, Heurkens AH, Booma-Frankfort C, Borg EJ et al. Five-year followup of rheumatoid arthritis patients after early treatment with disease-modifying antirheumatic drugs versus treatment according to the pyramid approach in the first year. Arthritis Rheum 2003; 48(7):1797-1807.
13. Grigor C, Capell H, Stirling A, McMahon AD, Lock P, Vallance R et al. Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomised controlled trial. Lancet 2004; 364(9430):263-269.

14. Shanahan JC, Moreland LW, Carter RH. Upcoming biologic agents for the treatment of rheumatic diseases. Curr Opin Rheumatol 2003; 15(3):226-236.
15. Keystone EC, Kavanaugh AF, Sharp JT, Tannenbaum H, Hua Y, Teoh LS et al. Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial. Arthritis Rheum 2004; 50(5):1400-1411.
16. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. N Engl J Med 2000; 343(22):1594-1602.
17. Klareskog L, van der Heijde D., de Jager JP, Gough A, Kalden J, Malaise M et al. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. Lancet 2004; 363(9410):675-681.
18. Furst DE, Breedveld FC, Kalden JR, Smolen JS, Burmester GR, Bijlsma JW et al. Updated consensus statement on biological agents, specifically tumour necrosis factor alpha (TNFalpha) blocking agents and interleukin-1 receptor antagonist (IL-1ra), for the treatment of rheumatic diseases, 2004. Ann Rheum Dis 2004; 63:Suppl-ii12.
19. Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS et al. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet 1994; 344(8930):1105-1110.
20. Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M et al. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. Lancet 1999; 354(9194):1932-1939.
21. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Furst D, Weisman MH et al. Sustained improvement over two years in physical function, structural damage, and signs and symptoms among patients with rheumatoid arthritis treated with infliximab and methotrexate. Arthritis Rheum 2004; 50(4):1051-1065.
22. Breedveld FC, Emery P, Keystone E, Patel K, Furst DE, Kalden JR et al. Infliximab in active early rheumatoid arthritis. Ann Rheum Dis 2004; 63(2):149-155.
23. St Clair EW, van der Heijde DM, Smolen JS, Maini RN, Bathon JM, Emery P et al. Combination of infliximab and methotrexate therapy for early rheumatoid arthritis: a randomized, controlled trial. Arthritis Rheum 2004; 50(11):3432-3443.
24. Taylor PC, Steuer A, Gruber J, Cosgrove DO, Blomley MJ, Marsters PA et al. Comparison of ultrasonographic assessment of synovitis and joint vascularity with radiographic evaluation in a randomized, placebo-controlled study of infliximab therapy in early rheumatoid arthritis. Arthritis Rheum 2004; 50(4):1107-1116.
25. Conaghan PG, Quinn MA, O'Connor P, Wakefield RJ, Karim Z, Emery P. Can very high-dose anti-tumor necrosis factor blockade at onset of rheumatoid arthritis produce long-term remission? Arthritis Rheum 2002; 46(7):1971-1972.

26. Quinn MA, Conaghan PG, O'Connor PJ, Karim Z, Greenstein A, Brown A et al. Very early treatment with infliximab in addition to methotrexate in early, poor-prognosis rheumatoid arthritis reduces magnetic resonance imaging evidence of synovitis and damage, with sustained benefit after infliximab withdrawal: results from a twelve-month randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2005; 52(1):27-35.
27. Moreland LW, Baumgartner SW, Schiff MH, Tindall EA, Fleischmann RM, Weaver AL et al. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. N Engl J Med 1997; 337(3):141-147.
28. Kremer JM, Weinblatt ME, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Jackson CG et al. Etanercept added to background methotrexate therapy in patients with rheumatoid arthritis: continued observations. Arthritis Rheum 2003; 48(6):1493-1499.
29. Van der Heijde D, Klareskog L, Wajdula J, Pedersen R, Fatenajad S for the TEMPO study investigators. Halting of radiographic progression in RA patients treated with Etanercept and methotrexate; year 2 results from the TEMPO Trial. Arthritis Rheum 2004; 50(12):4098.
30. Klareskog L, Van der Heijde D, Wajdula J, Pedersen R, Perkins P, Fatenajad S for the TEMPO study investigators. Therapeutic responsefollowing two years of treatment with etanercept, methotrexate, or combination therapy in patients with Early Rheumatoid Arthritis (RA). Arthritis Rheum 2004; 50(9):s238.
31. Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC et al. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. N Engl J Med 2000; 343(22):1586-1593.
32. Genovese MC, Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH et al. Etanercept versus methotrexate in patients with early rheumatoid arthritis: two-year radiographic and clinical outcomes. Arthritis Rheum 2002; 46(6):1443-1450.
33. Genovese MC, Martin R W, Fleischmann R M, Keystone E C, Bathon J M, Finck B et al. Etanercept (Enbrel) in Early Erosive Rheumatoid Arthritis (ERA Trial): Observations at 3 Years. Arthritis Rheum 2001; 44:s78.
34. Genovese MC, Martin R W, Fleischmann R M, Keystone E C, Bathon J M, Spencer-Green G T. Etanerecept (Enbrel) in Early Erosive Rhuematoid Atrthritis (ERA Trial): Observations at 4 Years. Arthritis Rheum 2002; 46:s 530.
35. Baumgartner SW, Fleischmann RM, Moreland LW, Schiff MH, Markenson J, Whitmore JB. Etanercept (Enbrel) in patients with rheumatoid arthritis with recent onset versus established disease: improvement in disability. J Rheumatol 2004; 31(8):1532-1537.
36. Moreland LW, Cohen SB, Baumgartner SW, Tindall EA, Bulpitt K, Martin R et al. Long-term safety and efficacy of etanercept in patients with rheumatoid arthritis. J Rheumatol 2001; 28(6):1238-1244.
37. Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA et al. Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum 2003; 48(1):35-45.
38. van de Putte LB, Atkins C, Malaise M, Sany J, Russell AS, van Riel PL et al. Efficacy and safety of adalimumab as monotherapy in patients with rheumatoid arthritis for whom previous disease modifying antirheumatic drug treatment has failed. Ann Rheum Dis 2004; 63(5):508-516.

39. Breedveld F C, Kavanaugh A F, Cohen S B, Pavelka K, Van Vollenhove R, Perez J L et al. Early treatment of rheumatoid arthritis (RA) with adalimumab (HUMIRA) pLus methotrexate vs. adalimumab alone or methotrexate alone: The Premier Study. Arthritis Rheum 2004; 50(12):4096.
40. Emery P, Kalden J R, Spencer-Green G T, Segurado O G. Adalimumab (HUMIRA) plus methotrexate induces sustained remission in both early and long- standing rheumatoid arthritis. Arthritis Rheum 2004; 50(9):S183.
41. Wolfe F, Michaud K. Lymphoma in rheumatoid arthritis: the effect of methotrexate and anti-tumor necrosis factor therapy in 18,572 patients. Arthritis Rheum 2004; 50(6):1740-1751.
42. Kavanaugh A, Keystone E C. The safety of biologic agents in early rheumatoid arthritis. experimental rheumatology 2003; 21(suppl 31):s203-s208.
43. Fleischmann R, Yocum D. Does safety make a difference in selecting the right TNF antagonist? Arthritis Res Ther 2004; 6:Suppl-8.
44. Goekoop-Ruiterman YPM, De Vries-Bouwstra JK, Van Zeben D, Kerstens PJSM, Hazes JMW, Zwinderman AH et al. Treatment strategies in early rheumatoid arthritis: clinical and radiological outcomes after 2 year follow-up of the BeSt study. Arthritis Rheum 2004; 50(12):4096.

Chapter 4

Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study)

Y. P. M. Goekoop-Ruiterman *
J. K. de Vries-Bouwstra *
C. F. Allaart
D. van Zeben
P. J. S. M. Kerstens
J. M. W. Hazes
A. H. Zwinderman
H. K. Ronday
K. H. Han
M. L. Westedt
A. H. Gerards
J. H. L. M. van Groenendael
W. F. Lems
M. V. van Krugten
F. C. Breedveld
B. A. C. Dijkmans

Arthritis Rheum 2005; 52 (11): 3381-90.

* Both authors contributed equally to this study

ABSTRACT

Objective Several treatment strategies have proven value in the amelioration of rheumatoid arthritis (RA), but the optimal strategy for preventing long-term joint damage and functional decline is unclear. We undertook this study to compare clinical and radiographic outcomes of four different treatment strategies, with intense monitoring in all patients.

Methods In a multicenter randomized clinical trial, 508 patients were allocated to one of four treatment strategies: sequential disease-modifying antirheumatic drug monotherapy (group 1), step-up combination therapy (group 2), initial combination therapy with tapered high-dose prednisone (group 3), and initial combination therapy with the tumor necrosis factor-α antagonist infliximab (group 4). Treatment adjustments were made every 3 months in an effort to obtain low disease activity (Disease Activity Score in 44 joints (DAS) ≤ 2.4).

Results Initial combination therapy including either prednisone (group 3) or infliximab (group 4) resulted in earlier functional improvement than did sequential monotherapy (group 1) and step-up combination therapy (group 2), with mean scores at 3 months on the Dutch version of the Health Assessment Questionnaire (HAQ) of 1.0 in groups 1 and 2 and 0.6 in groups 3 and 4 ($P < 0.001$). After 1 year, mean HAQ scores were 0.7 in groups 1 and 2 and 0.5 in groups 3 and 4 ($P = 0.009$). The median increase in total Sharp-van der Heijde radiographic joint score were 2.0, 2.5, 1.0, and 0.5 in groups 1 - 4, respectively ($P < 0.001$). There were no significant differences in the number of adverse events and withdrawals between the groups.

Conclusion In patients with early RA, initial combination therapy including either prednisone or infliximab resulted in earlier functional improvement and less radiographic damage after one year than did sequential monotherapy or step-up combination therapy.

INTRODUCTION

Over the last two decades, the treatment of patients with rheumatoid arthritis (RA) has changed considerably. Currently, the goal of therapy is not only symptom relief, but in particular the prevention of long-term structural damage and functional decline. To this end, an increasing number of effective disease modifying antirheumatic drugs (DMARDs) as well as biologic agents have been developed and have demonstrated clinical value in randomized clinical trials. It has become clear that treatment should start early and must be maintained without interruption to reduce the occurrence of irreversible joint damage (1-8). Furthermore, several combinations of DMARDs as well as tumor necrosis factor (TNF)-α antagonists have shown superiority to DMARD monotherapy in patients with early (9-17) and long-standing (18-22) RA. Finally, intensive monitoring of disease activity and adjusting DMARD use accordingly has resulted in improved outcomes (23). However, the increase in therapeutic options has left unanswered the question of what the optimal therapeutic strategy is in patients presenting with RA.

The BeSt (Dutch acronym for Behandel Strategieën, ''treatment strategies'') study is a multicenter randomized clinical trial in which we compared the clinical and radiographic outcomes of four different treatment strategies: sequential monotherapy (group 1), step-up combination therapy (group 2), initial combination therapy with tapered high-dose prednisone (group 3), and initial combination therapy with the TNF-α antagonist infliximab (group 4). The common goal in all strategies was to reduce disease activity rapidly and persistently by tight monitoring and immediate adjustment of therapy in case of an insufficient response. Here we present the results of the first year of follow-up.

PATIENTS AND METHODS

Patients

The BeSt study was designed and conducted by rheumatologists participating in the Foundation for Applied Rheumatology Research (FARR) in 18 peripheral and 2 university hospitals in the Western part of The Netherlands. The Medical Ethics Committee at each participating center approved the study protocol, and all patients gave written informed consent before inclusion. Patients with early RA, as defined by the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 revised criteria (24), were recruited between April 2000 and August 2002. Patients had to have a disease duration less than 2 years, be age of ≥ 18 years, and have active disease with ≥ 6 of 66 swollen joints, ≥ 6 of 68 tender joints, and either an erythrocyte sedimentation rate (ESR) of ≥ 28 mm/hour or a global health score of ≥ 20 mm on a 0–100 mm visual analog scale, where 0 = best and 100 = worst. Exclusion criteria included previous treatment with DMARDs other than antimalarials, concomitant treatment with an experimental

Figure 1. The four treatment strategies compared in the BeSt study

GROUP 1 SEQUENTIAL MONOTHERAPY	GROUP 2 STEP-UP COMBINATION THERAPY
MTX 15 mg/wk	MTX 15 mg/wk
↓	↓
MTX 25 mg/wk	MTX 25 mg/wk
↓	↓
SSZ 1000 mg bid	MTX 25 mg/wk + SSZ 1000 mg bid
↓	↓
leflunomide 20 mg/day	MTX 25 mg/wk + SSZ 1000 mg bid + HCQ 200 mg bid
↓	↓
MTX 25 mg/wk + infliximab 3 mg/kg/8 wks‡	MTX 25 mg/wk + SSZ 1000 mg bid + HCQ 200 mg bid + prednisone 7.5 mg/day
↓	↓
MTX 25 mg/wk + infliximab 6 mg/kg/8 wks‡	MTX 25 mg/wk + infliximab 3 mg/kg/8 wks‡
↓	↓
MTX 25 mg/wk + infliximab 7.5 mg/kg/8 wks‡	MTX 25 mg/wk + infliximab 6 mg/kg/8 wks‡
↓	↓
MTX 25mg/wk + infliximab 10mg/kg/8 wks‡	MTX 25 mg/wk + infliximab 7.5 mg/kg/8 wks‡
↓	↓
gold 50 mg/wk + 3x depomedrol 120 mg (week 1, 4, 8)	MTX 25 mg/wk + infliximab 10 mg/kg/8 wks‡
↓	↓
MTX 25 mg/wk + CSA 2.5 mg/kg/day + prednisone 7.5 mg/day	MTX 25 mg/wk + CSA 2.5 mg/kg/day + prednisone 7.5 mg/day
	↓
	leflunomide 20 mg/day

MTX = methotrexate, SSZ = sulphasalazine, CSA = cyclosporine A, AZA = azathioprine, HCQ = hydroxychloroquine.
In all groups: 1 month overlap when switching from one DMARD to the next, MTX up to 30 mg/wk allowed if patient weighed > 80kg and SSZ up to 1500 mg bid allowed.
†Prednisone 60 mg/day in week 1, weekly dose reductions (40, 25, 20, 15, 10 mg/day) to 7.5 mg/day from week 7. If DAS ≤ 2.4, prednisone was tapered to nil starting at week 28 and MTX was tapered to nil starting at week 40. If DAS > 2.4, MTX was increased to 25 mg/wk and prednisone was continued until DAS ≤ 2.4, after which prednisone and MTX were tapered subsequently. If DAS > 2.4 after stopping MTX, the drug was reintroduced. If DAS > 2.4 after tapering prednisone, this was reintroduced (7.5 mg/day) and simultaneously MTX was increased to 25 mg/wk.

Figure 1. The four treatment strategies compared in the BeSt study

GROUP 3 INITIAL COMBINATION with PREDNISONE	GROUP 4 INITIAL COMBINATION with INFLIXIMAB
MTX 7.5 mg/wk + SSZ 1000 mg bid + prednisone 60→7.5 mg/day†	MTX 25 mg/wk + infliximab 3 mg/kg/8 wks‡
↓	↓
MTX 25 mg/wk + SSZ 1000 mg bid + prednisone 7.5 mg/day	MTX 25 mg/wk + infliximab 6 mg/kg/8 wks‡
↓	↓
	MTX 25 mg/wk + infliximab 7.5 mg/kg/8 wks‡
MTX 25 mg/wk + CSA 2.5 mg/kg/day + prednisone 7.5 mg/day	↓
	MTX 25 mg/wk + infliximab 10 mg/kg/8 wks‡
↓	↓
MTX 25 mg/wk + infliximab 3 mg/kg/8 wks‡	SSZ 1000 mg bid
↓	↓
MTX 25 mg/wk + infliximab 6 mg/kg/8 wks‡	leflunomide 20 mg/day
↓	
MTX 25 mg/wk + infliximab 7.5 mg/kg/8 wks‡	↓
↓	MTX 25 mg/wk + CSA 2.5 mg/kg/day + prednisone 7.5 mg/day
MTX 25 mg/wk + infliximab 10 mg/kg/8 wks‡	
↓	↓
leflunomide 20 mg/day	gold 50 mg/wk + 3x depomedrol 120 mg (week 1, 4, 8)
↓	
gold 50 mg/wk + 3x depomedrol 120 mg (week 1, 4, 8)	↓
↓	AZA 2-3 mg/kg/day + prednisone 7.5 mg/day
AZA 2-3 mg/kg/day + prednisone 7.5 mg/day	

Prednisone was reintroduced only once, if DAS >2.4 after one reintroduction the patient proceeded to the next step.
‡Infliximab 3 mg/kg at t = 0 wks, t = 2 and t = 6 wks and every 8 wks thereafter. Extra DAS calculations for dose adjustments were performed every 8 weeks, within one week before the next infusion of infliximab. If DAS > 2.4, the dose of the next infusion was subsequently increased to 6, 7.5 and finally 10 mg/kg/8wks. If DAS ≤ 2.4 for ≥ 6 months, the dose was reduced every next infusion until stopped.

drug, a malignancy within the last five years, bone marrow hypoplasia, a serum aspartate aminotransferase or alanine aminotransferase level (ALT) > 3 times the upper limit of normal, a serum creatinine level > 150 µmoles/liter or an estimated creatinine clearance < 75 ml/minute, diabetes mellitus, alcohol or drug abuse, concurrent pregnancy, wish to conceive during the study period, or inadequate contraception.

Treatment allocation and intervention

Patients were allocated to one of four treatment groups by variable block (9-13) randomization, stratified per center. Closed envelopes containing the patient study number, the allocated treatment group, and preprinted prescriptions for the allocated treatment were distributed and stored by ascending stratified randomization numbers in the participating centers. After receiving authorization by telephone from the study coordinator, the local rheumatologists enrolled eligible patients.

Patients received sequential monotherapy (group 1), step-up combination therapy (group 2), initial combination therapy with tapered high-dos prednisone (group 3), or initial combination therapy with infliximab (group 4). For all groups, the treatment protocol described a number of subsequent treatment steps for patients whose medication failed (Figure 1). The decision of whether to adjust medication was made every 3 months based on the Disease Activity Score in 44 joints (DAS), which was calculated by a research nurse who remained blinded to the allocated treatment group during the entire study period. If the patient did not reach a DAS ≤ 2.4, the treating physician immediately adjusted therapy by proceeding to the next step in the allocated treatment group. If the clinical response was consistently adequate (DAS of ≤ 2.4 for at least 6 months), medication was gradually tapered until one drug remained in a maintenance dose. The DAS cut-off level of 2.4 was chosen because observational studies have shown that rheumatologists are generally satisfied with the treatment results and do not intensify therapy if the DAS is ≤ 2.4 (25,26).

The patients assigned to sequential monotherapy (group 1) started with 15 mg/wk methotrexate (MTX), which was increased to 25-30 mg/wk if the DAS was > 2.4. Subsequent steps for patients with an insufficient response were sulphasalazine (SSZ) monotherapy, leflunomide monotherapy, MTX with infliximab, gold with methylprednisolone, and, finally, MTX with cyclosporine A (CSA) and prednisone (Figure 1).

The patients assigned to step-up combination therapy (group 2) also started with 15 mg/wk MTX, which was increased to 25-30 mg/wk if the DAS was > 2.4. If response to therapy was still insufficient, SSZ was added, followed by the addition of hydroxychloroquine (HCQ) and then by prednisone. Patients whose disease failed to respond to the combination of these 4 drugs subsequently switched to MTX with infliximab, MTX with CSA and prednisone, and, finally, to leflunomide (Figure 1).

The patients assigned to initial combination therapy with prednisone (group 3) started with a combination of 7.5 mg/wk MTX, 2,000 mg/day SSZ, and 60 mg/day prednisone (the last of which was tapered in 7 weeks to 7.5 mg/day). In the case of a DAS of > 2.4, MTX was augmented to 25-30 mg/wk, and if the response was still insufficient,

the combination was replaced subsequently by the combination of MTX with CSA and prednisone, followed by MTX with infliximab, leflunomide monotherapy, gold with methylprednisolone, and, finally, by azathioprine (AZA) with prednisone. In the case of a persistent DAS of ≤ 2.4, first prednisone was tapered to zero after 28 weeks, and then MTX was tapered to zero after 40 weeks (Figure 1).

The patients assigned to the initial combination with infliximab started with 25-30 mg/wk MTX with 3 mg/kg infliximab at weeks 0, 2, and 6 and every 8 weeks thereafter. After 3 months, the dose of infliximab was increased to 6 mg/kg/every 8 weeks if the DAS was > 2.4. Extra DAS calculations for dose adjustments were performed every 8 weeks within 1 week before the next infusion of infliximab. If the DAS was > 2.4, the dose of the next infusion was increased to 7.5 mg/kg/every 8 weeks and finally to 10 mg/kg/every 8 weeks. If patients still had a DAS of > 2.4 while receiving MTX with 10 mg/kg infliximab, medication was subsequently switched to SSZ, then to leflunomide, then to the combination of MTX, CSA, and prednisone, then to gold with methylprednisolone, and, finally, to AZA with prednisone. In the case of persistent good response (DAS of ≤ 2.4 for at least 6 months), the dose of infliximab was reduced (from 10 to 7.5, 6, and then 3 mg/kg) every next infusion until stopped (Figure 1).

An overlap period of one month was used when switching from single DMARD to the next. Unless otherwise specified, the doses of the different drugs were as follows: for MTX, 25-30 mg/wk (oral or subcutaneous); for SSZ, 2,000-3,000 mg/day; for leflunomide, 20 mg/day; for HCQ, 400 mg/day; for prednisone, 7.5 mg/day; for CSA, 2.5 mg/kg/day; for gold, 50 mg/wk (intramuscular) with 120 mg methylprednisolone (intramuscular) at weeks 0, 4, and 8; for AZA, 2-3 mg/kg/day; and, for infliximab, 3-10 mg/kg/every 8 weeks (intravenous), as described above in greater detail for group 4.

In all groups, if the clinical response was consistently adequate (DAS of ≤ 2.4 for at least 6 months), drugs were tapered to monotherapy at a maintenance dose, which was 10 mg/wk for MTX, 2,000 mg/day for SSZ, 10 mg every other day for leflunomide, 50 mg every other week for gold, or 2 mg/kg/day for AZA. Prednisone and infliximab were always the first drugs to be tapered to a dose of zero. If disease activity flared (DAS > 2.4) after tapering a drug, the last effective dose was reintroduced. In all groups, prednisone could be reintroduced only once: if, after a second discontinuation, the DAS increased again to > 2.4, then the next step in the protocol was taken. Infliximab could be discontinued only once; after reintroduction, it could be tapered again, but only to a maintenance dose of 3 mg/kg/every 8 weeks. If side effects occurred, the responsible drug was reduced to the lowest tolerated dose. If a drug was not tolerated at all or contraindicated, patients receiving monotherapy proceeded to the next step in the allocated treatment group, and patients receiving combination therapy proceeded with the other drug(s) of the combination.

Contraindications for treatment with infliximab included the following: a known allergy to murine proteins, a chronic infectious disease, serious infections which occurred within the last 3 months, opportunistic infections which occurred within the last 6 months, a neurologic or cerebral disease, a lymphoproliferative disease, active tuberculosis (TB) within the last 2 years, and evidence of an old or latent TB infection for which latent TB therapy (isoniazid [INH]-based therapy or another regimen recommended by local

experts) was not instituted prior to infliximab therapy. Prior to infliximab therapy, all patients were evaluated for TB with a purified protein derivative skin test and a chest radiograph. At the beginning of 2002, heart failure was added as a contraindication for treatment with infliximab. Previously enrolled patients with heart failure who had already received infliximab continued therapy and were closely monitored.

Concomitant treatment with non steroidal antiinflammatory drugs and intraarticular injections with corticosteroids were permitted. Other parenteral corticosteroids were not allowed. The use of DMARDs or oral corticosteroids was only permitted as dictated by the treatment protocol. All patients received 1 mg/day folic acid during treatment with MTX.

Assessment of end points

Every 3 months, assessments were performed by a research nurse who was blinded to the allocated treatment group.

Primary end points were functional ability, measured by the Dutch version of the Health Assessment Questionnaire (HAQ) (27), and radiographic joint damage according to the modified Sharp-van der Heijde Score (SHS), with a range of 0-448 (28), assessed on radiographs of the hands and the feet obtained at baseline and after one year of follow-up. All radiographs were read by 2 trained assessors who were blinded for the patient's identity, treatment center, and date of radiograph and who scored the radiographs paired, in random order, and independently. The intraobserver coefficients were 0.93 and 0.94, and the interobserver coefficient was 0.93. The mean score of the two assessors was used for the analysis. A patient was classified as having erosive disease if the mean erosion score was > 0.5. Progression of radiographic joint damage was defined as a change in radiographic score greater than the smallest detectable difference (SDD), as well as by a change (in the total radiographic score) > 0.5 (29,30). The SDD was 5.92, 3.76, and 3.75 for total SHS, erosion score, and joint space narrowing score, respectively.

Secondary end points were 20%, 50%, and 70% improvement according to the ACR response criteria (31) and clinical remission, defined as a DAS of < 1.6 (32).

To maintain uniformity in scoring and assessment quality, all research nurses were trained at study initiation and every six months thereafter. Two trial physicians verified adherence to the protocol every three months. All protocol deviations were recorded.

Toxicity

At each control visit, the following laboratory tests were performed: ESR, complete blood cell count, and serum levels of ALT, gamma glutamyl transpeptidase, bilirubin, lactate dehydrogenase, creatinine, electrolytes, and glucose. The treating physician recorded all adverse events (AEs), serious AEs, and, if necessary, made treatment adjustments in accordance to the protocol. Serious AEs were defined as any adverse reaction resulting in any of the following outcomes: a life-threatening condition or death, a significant or permanent disability, a malignancy, hospitalization or prolongation of hospitalization, a congenital abnormality, or a birth defect.

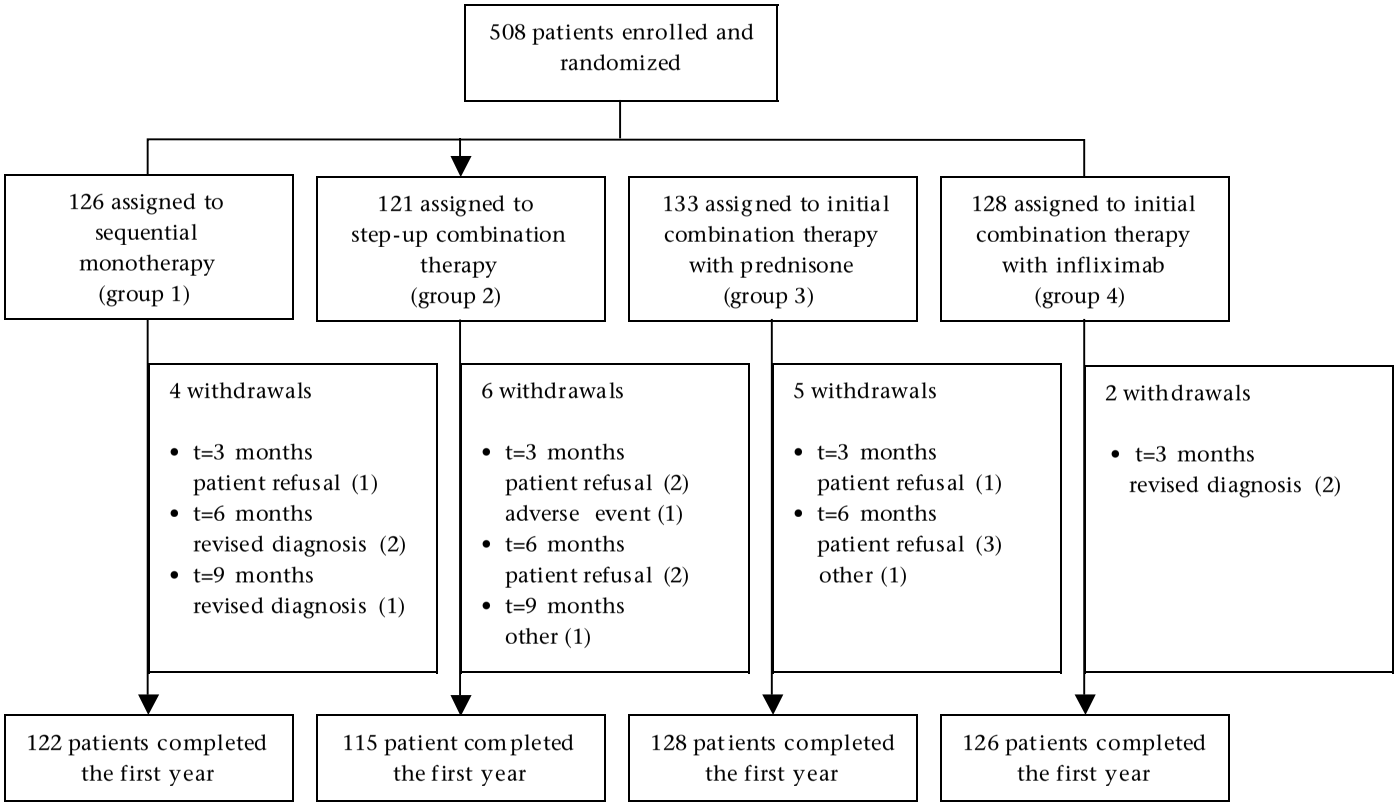


Figure 2. Study profile
Revised diagnoses were paraneoplastic arthritis, gout, systemic lupus erythemathosus, mixed connective tissue disease and Henoch-Schönlein purpura.

Statistical analysis

A total sample size of 468 patients (117 per group) was needed to obtain 80% power to detect a difference of at least 0.2 in HAQ score, which was set as a clinically relevant difference, with a 5% significance level and adjusting for multiple comparison between groups, assuming an SD of 0.45. This sample size also ensured > 80% power to detect a difference of ≥ 20% in the change score of radiographic damage as measured by the SHS.

All outcomes were calculated in an intention-to-treat analysis (ITT) using all available data. Measures with a Gaussian distribution, expressed as mean and standard deviation (SD), were analyzed using a one-way analysis of variance. In the case of an overall significant difference between the groups, a post-hoc least significant difference test was performed for the primary outcomes, and Tukey's honestly significant difference test was used for the secondary outcomes to correct for multiple testing. Outcome measurements with a non-Gaussian distribution, expressed as the median and interquartile range (IQR), were analyzed by the Kruskal-Wallis test. Pairwise comparisons between groups were performed using the Mann-Whitney U test. For the SHS, the change scores were reported both as the mean and as the median. Categorical variables such as sex and rheumatoid factor (RF) positivity were compared between treatment groups using the chi-square test. A subgroup analysis of the progression of radiographic joint damage was performed in patients who either did or did not have erosive disease at baseline.

RESULTS

Five hundred eight patients were included and randomly assigned to one of four treatment groups: 126 patients were assigned to sequential monotherapy (group 1), 121 patients to step-up combination therapy (group 2), 133 patients to initial combination therapy including prednisone (group 3), and 128 patients to initial combination therapy including infliximab (group 4). Seventeen patients dropped out (4, 6, 5, and 2 patients in groups 1-4, respectively) (Figure 2). Twenty-four patients (5%) discontinued adherence to the protocol because of noncompliance (5, 8, 8, and 3 patients in groups 1-4, respectively), but these patients were not lost to follow-up, and all available data were included in the ITT analysis.

There were no statistically significant differences in the demographic and baseline disease characteristics of the four groups (Table 1). The study population consisted of patients with very early RA, with a median duration between diagnosis and inclusion of 2 weeks (IQR 1-5) and a median duration of symptoms of 23 weeks (IQR 14-53). All patients had active disease with a mean ± SD DAS of 4.4 ± 0.9, and 72% of the patients had erosive disease at baseline.

Two patients with latent TB in group 4 refused concomitant treatment with INH. By mistake, these patients started with the next treatment step (SSZ) instead of MTX monotherapy. Two patients were excluded from treatment with infliximab (1 because of

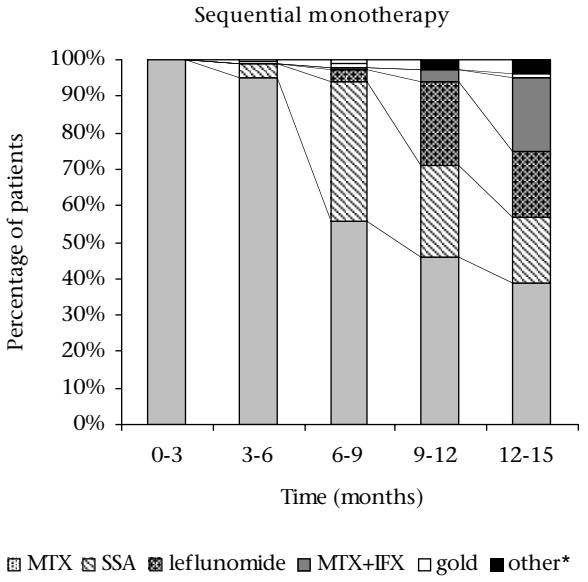
a history of untreated TB accompanied by a lesion on a chest radiograph and 1 because of cardiac failure) and started with MTX monotherapy according to the treatment protocol. All 4 patients were analyzed for treatment in group 4 according to the ITT principle.

The goal in each treatment group was to reach and sustain a DAS of ≤ 2.4, indicating low disease activity. After one year, this goal was reached by 63 of 118 patients (53%), 72 of 112 patients (64%), 87 of 121 patients (71%), and 89 of 121 patients (74%) in groups 1-4, respectively ($P = 0.004$ for group 1 versus group 3; $P = 0.001$ for group 1 versus group 4; P not significant [NS] for other comparisons). More patients in groups 3 and 4 than in groups 1 and 2 remained at the initial stage of treatment because of a sustained DAS of ≤ 2.4 (48 [39%], 43 [37%], 94 [74%], and 102 [81%] of the patients in groups 1-4, respectively) (Figure 3). Of these patients, 78% in group 3 had stopped prednisone and 50% in group 4 had stopped infliximab, because of a persistent DAS ≤ 2.4 (Figure 3). The number of patients who had received intraarticular steroids at least once was 28 (22%), 32 (26%), 10 (8%), and 17 (13%) in groups 1-4, respectively ($P = 0.001$).

Table 1. Baseline demographic and disease characteristics*

	Sequential monotherapy (n=126)	Step-up combination therapy (n=121)	Initial combination with prednisone (n=133)	Initial combination with infliximab (n=128)
Age, mean (SD) years	54 (13)	54 (13)	55 (14)	54 (14)
Women, no. (%)	86 (68)	86 (71)	86 (65)	85 (66)
Time from diagnosis to inclusion, median weeks (IQR)	2 (1-5)	2 (1-4)	2 (1-4)	3 (1-5)
Symptom duration, median weeks (IQR)	23 (14-54)	26 (14-56)	23 (15-53)	23 (13-46)
Previous use of antimalarials, no. (%)	9 (7)	13 (11)	10 (8)	11 (9)
IgM RF positive, no. (%)	84 (67)	77 (64)	86 (65)	82 (64)
DAS, mean (SD)	4.5 (0.9)	4.5 (0.8)	4.4 (0.9)	4.3 (0.9)
HAQ score, mean (SD)	1.4 (0.7)	1.4 (0.6)	1.4 (0.7)	1.4 (0.7)
Total SHS				
median (IQR)	3.5 (1.5-9.5)	5.0 (1.5-8.1)	3.5 (1.5-8.5)	4.0 (1.5-8.5)
mean (SD)	7.3 (9.5)	6.3 (6.9)	5.9 (6.5)	7.0 (10.0)
Erosion score				
median (IQR)	2.0 (0.5-4.5)	2.0 (0.5-4.5)	2.0 (0.5-4.5)	2.0 (0.5-5.0)
mean (SD)	4.1 (6.2)	3.5 (4.3)	3.3 (4.3)	3.9 (5.8)
Joint space narrowing score				
median (IQR)	1.0 (0.0-4.0)	2.0 (0.0-4.5)	1.5 (0.0-4.0)	1.5 (0.0-3.5)
mean (SD)	3.2 (4.9)	2.8 (3.2)	2.6 (3.2)	3.1 (5.2)
Erosions on hand/foot radiograph, no. (%)	89 (72)	82 (70)	93 (71)	93 (73)

* IQR = interquartile range; RF = rheumatoid factor DAS = Disease Activity Score in 44 joints; HAQ = Dutch version of Health Assessment Questionnaire; SHS = Sharp-van der Heijde score.



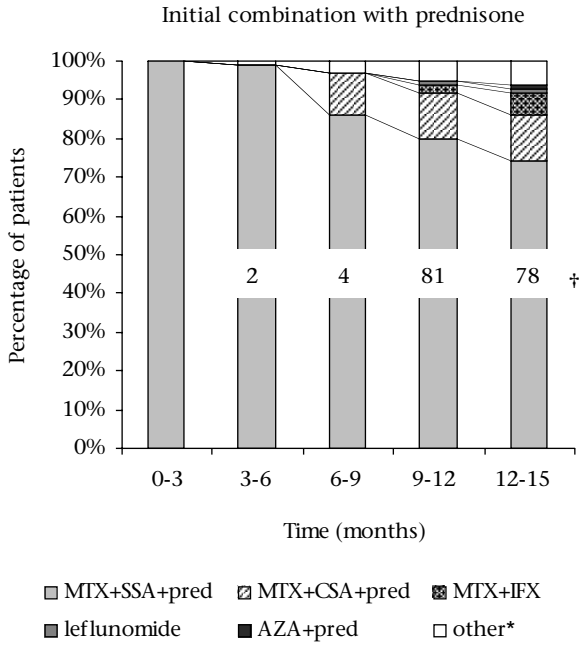
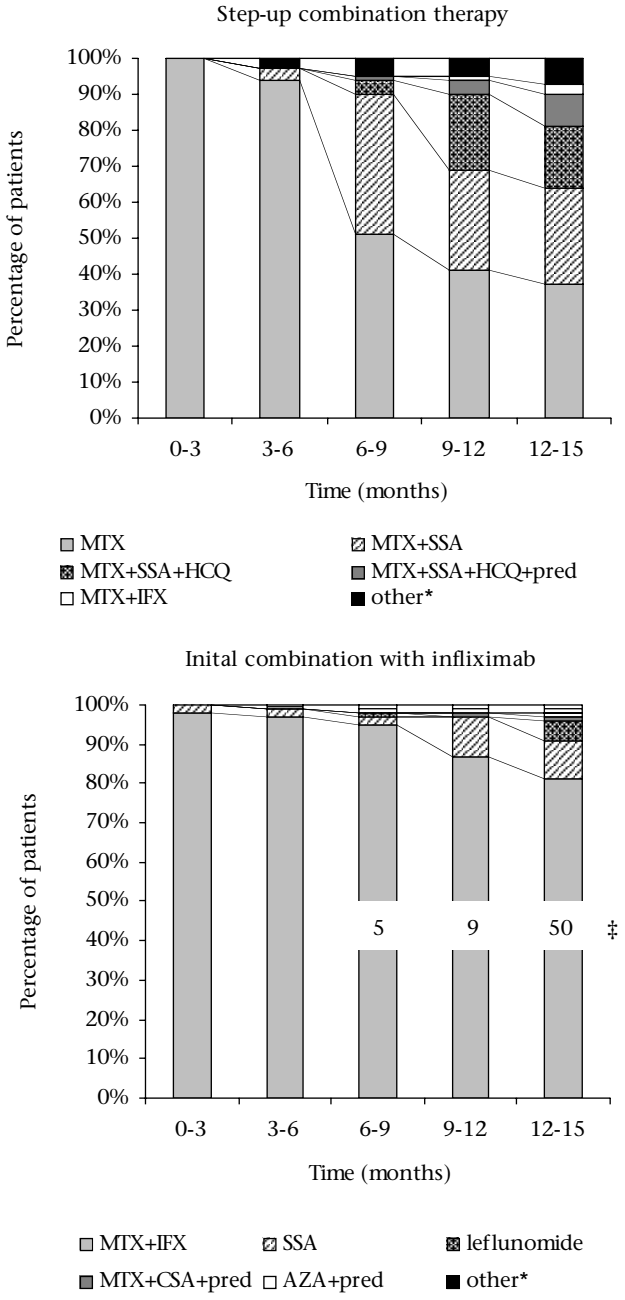


Figure 3. Treatment of patients during the first year of follow-up
* = percentage of patients treated outside the treatment protocol.
† = percentage of patients who discontinued prednisone, because of a sustained DAS ≤ 2.4.



‡ = percentage of patients who discontinued infliximab because of a sustained DAS ≤ 2.4.
MTX = methotrexate, SSA = sulphasalazine, IFX = infliximab, HCQ = hydroxychloroquine, pred = prednisone, CSA = cyclosporine A, AZA = azathioprine.

Clinical outcomes

Patients treated with initial combination therapy with either prednisone (group 3) or infliximab (group 4) had more rapid functional improvement than patients treated with sequential monotherapy (group 1) or step-up combination therapy (group 2) (Figure 4, Table 2). The mean HAQ score at 3 months was 1.0 in groups 1 and 2 and 0.6 in groups 3 and 4 ($P < 0.001$ for groups 1 and 2 versus groups 3 and 4; P NS for other comparisons). After one year, the differences in HAQ scores between the groups were smaller, with mean HAQ scores of 0.7 in groups 1 and 2 and of 0.5 in groups 3 and 4 ($P = 0.010$ for group 1 versus group 3; $P = 0.003$ for group 1 versus group 4; P NS for other comparisons). Thirty-two percent of all patients had clinical remission of their disease (DAS < 1.6) after the first year of follow-up (overall $P = 0.690$) (Figure 4). Clinical improvement, as defined by the ACR response criteria, was reached earlier and by more patients in groups 3 and 4 than in groups 1 and 2 (Figure 4).

Radiographic outcomes

At baseline, 499 radiographs were assessed (123, 118, 131, and 127 in groups 1-4, respectively). The treatment groups were similar at baseline with respect to the number of erosions, joint space narrowing and total SHS (Table 1). Radiographs obtained at baseline and at one year of follow-up were available for 467 patients. Compared with patients with baseline and follow-up radiographs, the 32 patients without follow-up radiographs (including the 17 who withdrew) had a higher total SHS with more erosions at baseline, but did not differ in baseline joint space narrowing, age, sex, RF positivity, HAQ score, DAS and ESR, and patients were equally distributed over the four treatment groups (data not shown).

In the first year of follow-up, patients treated with initial combination therapy including prednisone (group 3) or infliximab (group 4) had less progression of radiographic joint damage than did patients treated with sequential monotherapy (group1) or step-up combination therapy (group 2) (Table 2). The median increases in the total SHS were 2.0, 2.5, 1.0 and 0.5 in groups 1-4, respectively ($P = 0.003$ for group 1 versus group 3; $P < 0.001$ for group 1 versus group 4, $P = 0.007$ for group 2 versus group 3; $P < 0.001$ for group 2 versus group 4) (Table 2).

The number of patients without progression of radiographic joint damage was higher in groups 3 and 4 than in groups 1 and 2 (Figure 5). No progression of the total SHS (greater than the SDD) was observed in 76 of 114 patients (67%), 82 of 112 patients (74%), 104 of 120 patients (87%), and 113 of 121 patients (93%) in groups 1-4, respectively ($P < 0.001$ for group 1 versus groups 3 and 4; $P = 0.010$ for group 2 versus group 3; $P < 0.001$ for group 2 versus group 4; P NS for other comparisons). Improvement of the total SHS (greater than the SDD) was seen in 1 patient each in groups 1, 3, and 4. Of all patients with nonerosive disease at baseline, 9 of 31 (29%) in group 1, 18 of 34 (53%) in group 2, 14 of 37 (38%) in group 3, and 5 of 34 (15%) in group 4 progressed to erosive disease ($P = 0.050$ for group 1 versus group 2; $P = 0.001$ for group 2 versus group 4; $P = 0.028$ for group 3 versus group 4; P NS for other comparisons).

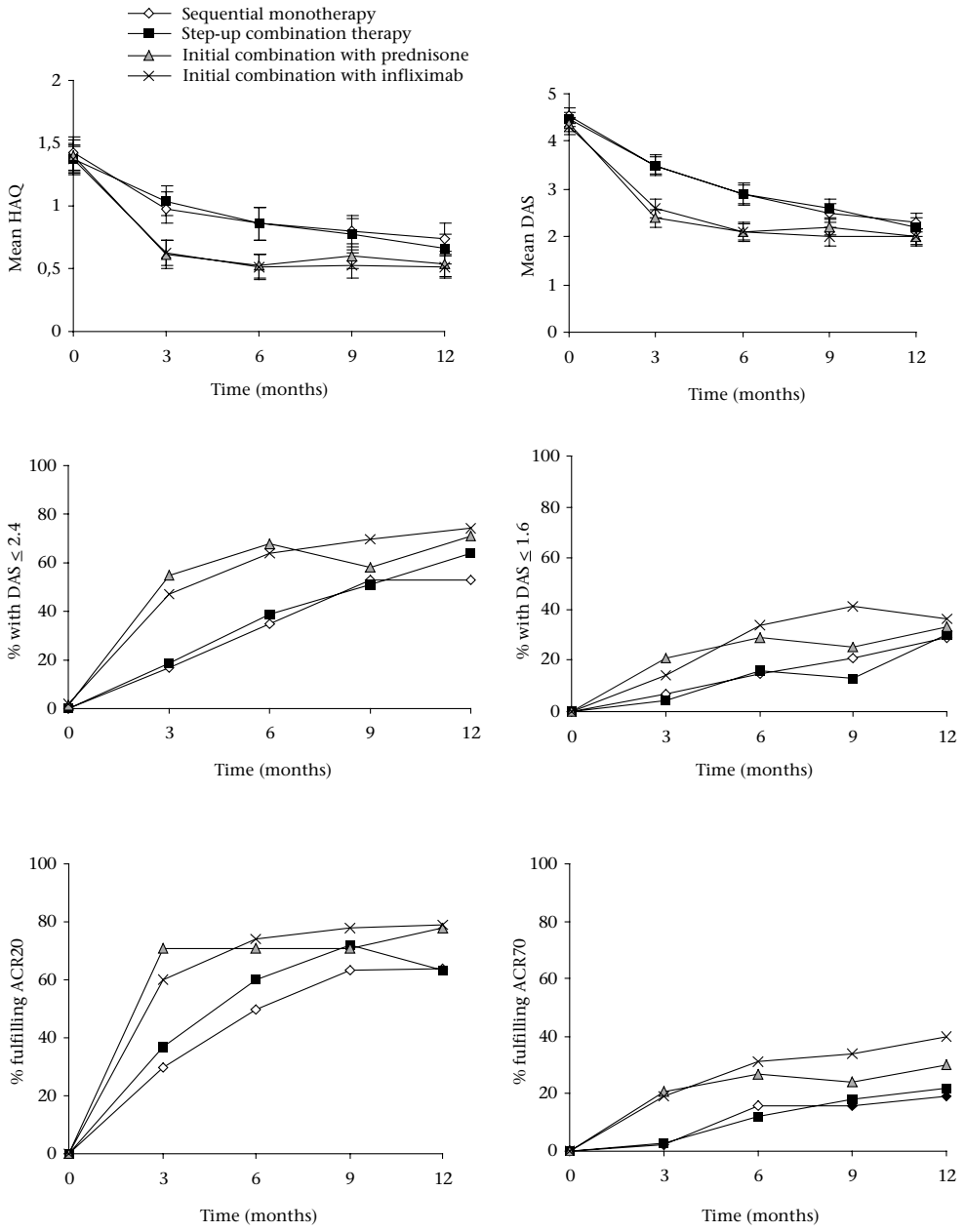


Figure 4. Clinical outcomes
Error bars indicate 95% confidence intervals.
HAQ = Dutch version of the Health Assessment Questionnaire; DAS = Disease Activity Score in 44 joints (DAS ≤ 2.4 indicates adequate clinical response; DAS < 1.6 indicates clinical remission); ACR20 = 20% improvement according to American College of Rheumatology response criteria.

Table 2. Primary outcomes of the BeSt study *

	Sequential monotherapy	Step-up combination therapy	Initial combination with prednisone	Initial combination with infliximab	*P*
HAQ score, mean ± SD					
Baseline	1.4 ± 0.7	1.4 ± 0.6	1.4 ± 0.7	1.4 ± 0.7	NS
3 months	1.0 ± 0.7	1.0 ± 0.6	0.6 ± 0.6	0.6 ± 0.6	<0.001†
6 months	0.9 ± 0.7	0.9 ± 0.7	0.5 ± 0.5	0.5 ± 0.5	<0.001†
9 months	0.8 ± 0.7	0.8 ± 0.7	0.6 ± 0.6	0.5 ± 0.6	0.001†
12 months	0.7 ± 0.7	0.7 ± 0.6	0.5 ± 0.5	0.5 ± 0.5	0.009‡
Progression of radiographic joint damage, median (IQR)/ mean ± SD					
Total SHS	2.0 (0.0-7.4)/ 7.1 ±15.4	2.5 (0.0-6.0)/ 4.3 ±6.5	1.0 (0.0-2.5)/ 2.0 ±3.6	0.5 (0.0-2.3)/ 1.3 ±4.0	<0.001†
Erosion score	1.0 (0.0-3.9)/ 3.5 ±8.2	1.0 (0.0-4.0)/ 2.6 ±4.7	0.5 (0.0-1.4)/ 0.9 ±1.9	0.0 (0.0-1.5)/ 0.7 ±2.1	<0.001†
Joint space narrowing score	1.0 (0.0-3.8)/ 3.6 ±8.4	0.0 (0.0-2.4)/ 1.6 ±2.9	0.0 (0.0-1.9)/ 1.0 ±2.4	0.0 (0.0-1.0)/ 0.6 ±2.6	<0.001§

NS = not significant (see Table 1 for other definitions).† $P < 0.05$, groups 1 and 2 versus groups 3 and 4. ‡ $P < 0.05$, group 1 versus groups 3 and 4. § $P < 0.05$ group 1 versus groups 3 and 4 and group 2 versus group 4.

Toxicity

A total of 41% of all patients experienced ≥ one AEs: 54 (43%), 57 (47%), 49 (37%), and 50 (39%) of the patients in groups 1-4, respectively (overall $P = 0.367$). Gastrointestinal symptoms were most frequently reported and were observed in 20 (16%), 18 (15%), 11 (8%), and 14 (11%) of the patients in group 1-4, respectively. Skin rash or other mild dermal or mucosal events were reported in 12 (10%), 15 (12%), 12 (9%), and 8 (6%) of the patients in group 1-4, respectively. Ten patients in group 4 had a mild-to-moderate infusion reaction during treatment with infliximab. Infliximab was discontinued in these patients. Nine patients in group 4 had latent TB and received concomitant INH prior to the initiation of infliximab therapy. No cases of TB or opportunistic infections were reported.

There were 8, 9, 17, and 6 serious AEs reported in groups 1-4, respectively ($P = 0.438$ for comparison of the number of patients with serious AEs between the treatment groups). In group 1, patients were hospitalized for the following reasons: 1 for hypertension, 1 for transient ischemic attack, 1 for pulmonary embolism, 1 for pneumonia, 1 for herpes simplex encephalitis, 1 for a hip prosthesis operation, 1 for fever associated with SSZ, and 1 for active arthritis with revision of diagnosis to gout. In group 2, patients were hospitalized for the following reasons: 1 for a peripheral bypass operation, 1 for pacemaker implantation, 1 for a prolapsed vertebral disk, 1 for neuropathy, 1 for a hip prosthesis operation, 1 for diffuse peritonitis, and 2 for exacerbations of RA, and there was 1 malignancy (bladder carcinoma). In group 3, patients were hospitalized for the following reasons: 3 for myocardial infarction, 1 for heart failure, 1 for oral herpes simplex infection, 1 for hip fracture, 1 for hip pain, 1 for granulocytopenia, 1 for urinary tract stone, 1 for temporal arteriitis, 2 for exacerbation

of RA, 1 for excision of benign microcalcifications viewed on mammography, and 2 for appendectomy, and there were 2 malignancies (1 breast cancer and 1 lymphoma). Finally, in group 4, patients were hospitalized for the following reasons: 1 for transient cardiac ischemia, 1 for pulmonary embolism, 1 for peripheral vascular disease, 1 for pneumonia, 1 for septic arthritis, and 1 for MTX pneumonitis.

DISCUSSION

In the BeSt study, the clinical and radiographic efficacies of four different treatment strategies for early RA were compared in the search for the optimal strategy to prevent long-term joint damage and functional decline.

Initial combination therapy including either prednisone (group 3) or infliximab (group 4) resulted in earlier functional improvement compared with sequential monotherapy (group 1) and step-up combination therapy (group 2). By the end of the first year, there was a marked improvement in all groups, with 32% of all patients having clinical remission of their disease (DAS < 1.6). Presumably, this result after one year was due to close monitoring with immediate treatment adjustments made in all patients who had a DAS > 2.4. To achieve an adequate clinical response (DAS ≤ 2.4), medications were altered more often in groups 1 and 2 than in groups 3 and 4. The absence of a difference between groups 1 and

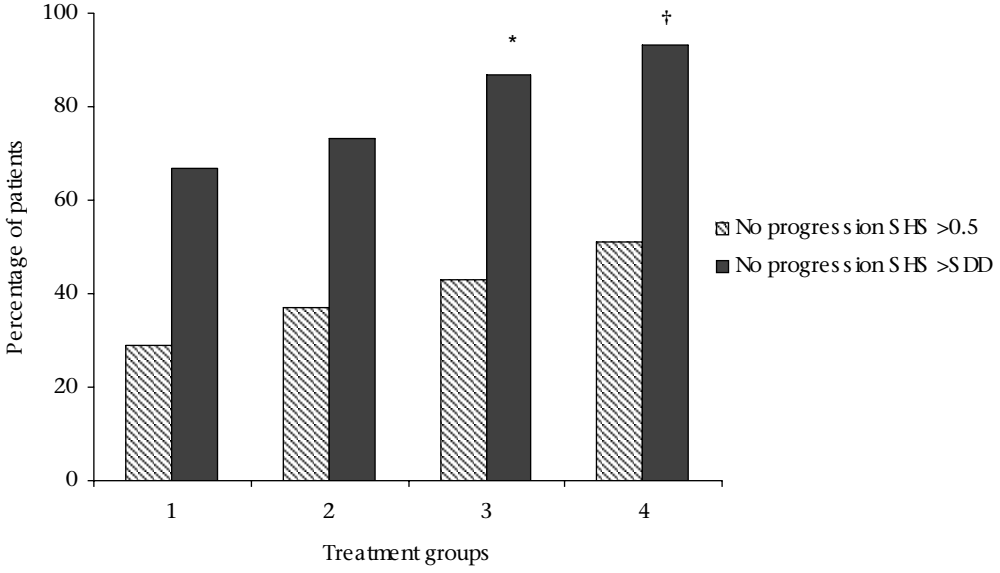


Figure 5. Percentage of patients without progression of radiographic joint damage
1 = sequential monotherapy; 2 = step-up combination therapy; 3 = initial combination therapy with prednisone; 4 = initial combination therapy with infliximab.
* = $P < 0.001$ versus group 1 and $P = 0.010$ versus group 2. † = $P < 0.001$ versus groups 1 and 2.
SHS = Sharp-van der Heijde Score. SDD = smallest detectable difference.

2 confirms observations that the combination of SSZ and MTX has no additive therapeutic effect (33,34) and suggests that with these 2 drugs, adding is not better than switching.

The four treatment strategies that were compared in the BeSt study are the most frequently used and discussed strategies. The group 1 strategy reflects conventional therapy in combination with tight disease control, which has recently been demonstrated to be more effective than routine care (23). The group 2 strategy was designed because the case for step-up combination therapy has not yet been proven. We chose to step up to the combination of MTX, SSZ, and HCQ with prednisone, which has been proven effective in previous studies (11,16,20). The group 3 strategy is designed according to the COBRA (Combinatietherapie Bij Reumatoïde Artritis) trial (9), and the group 4 strategy is considered to be the most aggressive strategy, with rapid dose increments of MTX in combination with the biologic agent infliximab. To minimize the risk of bias in the open design, all outcome measurements were assessed by trained research nurses who were blinded to the allocated treatment strategy during the entire study period, and the end points were chosen to allow for the least possible subjectivity of interpretation.

There were no statistically significant differences in the frequency of toxic effects and in the number of withdrawals between the four treatment groups. The difference in the progression of radiographic joint damage between the patients in groups 3 and 4 and the patients in groups 1 and 2 was statistically significant after one year of follow-up. From a clinical perspective, however, these differences were small. On the one hand, in > 40% of the patients in groups 1 and 2, a sustained adequate suppression of disease activity was achieved with MTX monotherapy, which is an indication that a large proportion of patients would be overtreated if all patients were to start with initial combination therapy. On the other hand, the patients in groups 3 and 4 had the benefit of a more rapid relief of symptoms and improvement of physical function. In addition, there is the possibility that effective suppression of disease activity during the early phases of the disease may ameliorate the long-term joint damage and poor physical function and, ideally, even induce true clinical remission without the need for DMARD treatment.

The follow-up of the COBRA study showed that the rate of progression of joint damage remained lower in the combination group for up to four years after the initial 56 week controlled intervention period (14). The same was seen in the early RA trial, in which patients treated with etanercept monotherapy had a more rapid clinical response and less progression of joint damage than patients treated with MTX (12). From this perspective, starting therapy with a single DMARD would be a missed opportunity in a considerable number of patients. The results of the long-term follow-up of the BeSt study, which includes analyses of joint destruction, physical function and cost-effectiveness, should clarify this issue. Furthermore, we hope to identify clinical and serologic parameters as well as genetic variations that can identify those patients who will benefit most from initial combination therapy.

In conclusion, during the first year of follow-up, patients with newly diagnosed RA who received initial combination therapy with either prednisone or infliximab had earlier functional improvement, with less progression of radiographic joint damage and fewer side effects than in patients who received sequential monotherapy or step-up combination therapy.

Contributors

All authors contributed to the study design, the analysis, the writing of this report and had access to all data and final responsibility for the decision to submit for publication. Y. P. M. Goekoop-Ruiterman and J. K. de Vries-Bouwstra were the trial coordinators, monitored adherence to the protocol and data collection in all participating centres and read radiographs; C. F. Allaart, B. A. C. Dijkmans and F. C. Breedveld were the principle investigators and part of the Steering Committee, as were P. J. S. M. Kerstens, D. van Zeben and J. M. W. Hazes; A. H. Zwinderman contributed to the statistical design and analysis; and H. K. Ronday, K. H. Han, M. L. Westedt, A. H. Gerards, J. H. L. M. van Groenendael, W. F. Lems and M. V. van Krugten were study centre coordinators at their respective centres.

Acknowledgments

We would like to thank all participating patients as well as the following rheumatologists (other than the authors) who participated in FARR (all locations are in The Netherlands): W. M. de Beus, MD (Medical Center Haaglanden, The Hague); M. H. W. de Bois, MD (Medical Center Haaglanden, The Hague); G. Collée, MD (Medical Center Haaglanden, The Hague); J. A. P. M. Ewals, MD (Haga Hospital, The Hague); B. A. M. Grillet, MD (De Honte Hospital, Terneuzen); H. M. J. Hulsmans, MD (Haga Hospital, The Hague); M. H. de Jager, MD (Albert Schweitzer Hospital, Dordrecht); J. M. de Jonge-Bok, MD (Groene Hart Hospital, Gouda); H. van der Leeden, MD (retired); M. F. van Lieshout-Zuidema, MD (Spaarne Hospital, Haarlem); A. Linssen, MD (Kennemer Gasthuis, Haarlem); P. A. H. M. van der Lubbe, MD (Vlietland Hospital, Schiedam); C. Mallée, MD (Kennemer Gasthuis, Haarlem); H. K. Markusse, MD (deceased); A. J. Peeters, MD (Reinier de Graaf Groep, Delft); D. van Schaardenburg, MD (VU Medical Center, Amsterdam and Jan van Breemen Institute, Amsterdam); P. E. H. Seys, MD (Lievensberg Hospital, Bergen op Zoom); R. M. van Soesbergen, MD (retired); P. B. J. de Sonnaville, MD (Oosterschelde Hospital, Goes); I. Speyer, MD (Bronovo Hospital, The Hague); J. Ph. Terwiel, MD (Spaarne Hospital, Haarlem); A. E. Voskuyl, MD (VU Medical Center, Amsterdam); S. ten Wolde, MD (Kennemer Gasthuis, Haarlem). We would also like to thank all other rheumatologists and trainee rheumatologists who enrolled patients in this study, and all research personnel for their contribution to this article.

Role of the funding source

This study was funded by a grant of the Dutch College of Health Insurances (College voor zorgverzekeringen) with additional funding provided by Schering-Plough, B.V. and Centocor, Inc. The authors were responsible for the study design, the collection, analysis and interpretation of all data, the writing of this article and the decision to publish.

REFERENCES

1. Bukhari MAS, Wiles NJ, Lunt M, Harrison BJ, Scott DGI, Symmons DPM et al. Influence of disease-modifying therapy on radiographic outcome in inflammatory polyarthritis at five years: results from a large observational inception study. Arthritis Rheum 2003; 48(1):46-53.
2. Egsmose C, Lund B, Borg G, Pettersson H, Berg E, Brodin U et al. Patients with rheumatoid arthritis benefit from early second line therapy: 5-year follow-up of a prospective double-blind placebo-controlled study. J Rheumatol 1995; 22(12):2208-2213.
3. Fries JF, Williams CA, Morfeld D, Singh G, Sibley J. Reduction in long-term disability in patients with rheumatoid arthritis by disease-modifying antirheumatic drug-based treatment strategies. Arthritis Rheum 1996; 39(4):616-622.
4. Lard LR, Visser H, Speyer I, vander Horst-Bruinsma IE, Zwinderman AH, Breedveld FC et al. Early versus delayed treatment in patients with recent-onset rheumatoid arthritis: comparison of two cohorts who received different treatment strategies. Am J Med 2001; 111(6):446-451.
5. Mottonen T, Hannonen P, Korpela M, Nissila M, Kautiainen H, Ilonen J et al. Delay to institution of therapy and induction of remission using single-drug or combination-disease-modifying antirheumatic drug therapy in early rheumatoid arthritis. Arthritis Rheum 2002; 46(4):894-898.
6. Sokka T, Mottonen T, Hannonen P. Disease-modifying anti-rheumatic drug use according to the ‚sawtooth' treatment strategy improves the functional outcome in rheumatoid arthritis: results of a long-term follow-up study with review of the literature. Rheumatology (Oxford) 2000; 39(1):34-42.
7. van der Heide A., Jacobs JW, Bijlsma JW, Heurkens AH, Booma-Frankfort C, van der Veen MJ et al. The effectiveness of early treatment with "second-line" antirheumatic drugs. A randomized, controlled trial. Ann Intern Med 1996; 124(8):699-707.
8. Fries JF. Reevaluating the therapeutic approach to rheumatoid arthritis: the "sawtooth" strategy. J Rheumatol Suppl 1990; 22:12-15.
9. Boers M, Verhoeven AC, Markusse HM, van de Laar MA, Westhovens R, van Denderen JC et al. Randomised comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis Lancet 1997; 350(9074):309-318.
10. Breedveld FC, Emery P, Keystone E, Patel K, Furst DE, Kalden JR et al. Infliximab in active early rheumatoid arthritis. Ann Rheum Dis 2004; 63(2):149-155.
11. Calguneri M, Pay S, Caliskaner Z, Apras S, Kiraz S, Ertenli I et al. Combination therapy versus monotherapy for the treatment of patients with rheumatoid arthritis. Clin Exp Rheumatol 1999; 17(6):699-704.
12. Genovese MC, Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH et al. Etanercept versus methotrexate in patients with early rheumatoid arthritis - Two-year radiographic and clinical outcomes. Arthritis Rheum 2002; 46(6):1443-1450.

13. Gerards AH, Landewe RB, Prins AP, Bruyn GA, Goei The HS, Laan RF et al. Cyclosporin A monotherapy versus cyclosporin A and methotrexate combination therapy in patients with early rheumatoid arthritis: a double blind randomised placebo controlled trial. Ann Rheum Dis 2003; 62(4):291-296.
14. Landewe RBM, Boers M, Verhoeven AC, Westhovens R, van de Laar MAFJ, Markusse HM et al. COBRA combination therapy in patients with early rheumatoid arthritis: long-term structural benefits of a brief intervention. Arthritis Rheum 2002; 46(2):347-356.
15. Marchesoni A, Battafarano N, Arreghini M, Panni B, Gallazzi M, Tosi S. Radiographic progression in early rheumatoid arthritis: a 12-month randomized controlled study comparing the combination of cyclosporin and methotrexate with methotrexate alone. Rheumatology (Oxford) 2003; 42(12):1545-1549.
16. Mottonen T, Hannonen P, Leirisalo-Repo M, Nissila M, Kautiainen H, Korpela M et al. and the FIN-RACo trial group. Comparison of combination therapy with single-drug therapy in early rheumatoid arthritis: a randomised trial. Lancet 1999; 353(9164):1568-1573.
17. St Clair EW, van der Heijde DM, Smolen JS, Maini RN, Bathon JM, Emery P et al. Combination of infliximab and methotrexate therapy for early rheumatoid arthritis: a randomized, controlled trial. Arthritis Rheum 2004; 50(11):3432-3443.
18. Klareskog L, van der HD, de Jager JP, Gough A, Kalden J, Malaise M et al. and the TEMPO (Trial of Etanercept and Methotrexate with Radiographic Ptient Outcomes) study investigators. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. Lancet 2004; 363(9410):675-681.
19. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR et al. and the Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. Infliximab and methotrexate in the treatment of rheumatoid arthritis. N Engl J Med 2000; 343(22):1594-1602.
20. O'Dell JR, Haire CE, Erikson N, Drymalski W, Palmer W, Eckhoff PJ et al. Treatment of rheumatoid arthritis with methotrexate alone, sulfasalazine and hydroxychloroquine, or a combination of all three medications. N Engl J Med 1996; 334(20):1287-1291.
21. Tugwell P, Pincus T, Yocum D, Stein M, Gluck O, Kraag G et al. The Methotrexate-Cyclosporine Combination Study Group. Combination therapy with cyclosporine and methotrexate in severe rheumatoid arthritis. N Engl J Med 1995; 333(3):137-141.
22. Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI et al. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N Engl J Med 1999; 340(4):253-259.
23. Grigor C, Capell H, Stirling A, McMahon AD, Lock P, Vallance R et al. Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomised controlled trial. Lancet 2004; 364(9430):263-269.
24. Arnett FC, Edworthy SM, Bloch DA, Mcshane DJ, Fries JF, Cooper NS et al. The American-Rheumatism-Association 1987 Revised Criteria for the Classification of Rheumatoid-Arthritis 2. Arthritis Rheum 1988; 31(3):315-324.

25. van der Heijde DM, Van 't Hof M, van Riel PLCM, Van de Putte LBA. Development of a disease activity score based on judgment in clinical practice by rheumatologists. J Rheumatol 1993; 20(3):579-581.
26. Van Gestel AM, Prevoo MLL, VantHof MA, VanRijswijk MH, VandePutte LBA, vanRiel PLCM. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis - Comparison with the preliminary American College of Rheumatology and the World Health Organization International League Against Rheumatism criteria. Arthritis Rheum 1996; 39(1):34-40.
27. Siegert CEH, Vleming LJ, Vandenbroucke JP, Cats A. Measurement of Disability in Dutch Rheumatoid-Arthritis Patients. Clin Rheumatol 1984; 3(3):305-309.
28. van der Heijde DM, van Riel PL, Gribnau FW, Nuverzwart IH, Vandeputte LB. Effects of hydroxychloroquine and sulphasalazine on progression of joint damage in rheumatoid arthritis. Lancet 1989; 1(8646):1036-1038.
29. Bland JM, Altman DG. Statistical methods for assessing agreement between 2 methods of clinical measurement. Lancet 1986; 1(8476):307-310.
30. Lassere M, Boers M, van der Heijde D, Boonen A, Edmonds J, Saudan A et al. Smallest detectable difference in radiological progression. J Rheumatol 1999; 26(3):731-739.
31. Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C et al. American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995; 38(6):727-735.
32. Prevoo MLL, VanGestel AM, VantHof MA, VanRijswijk MH, VandePutte LBA, vanRiel PLCM. Remission in a prospective study of patients with rheumatoid arthritis. American Rheumatism Association preliminary remission criteria in relation to the disease activity score. Br J Rheumatol 1996; 35(11):1101-1105.
33. Dougados M, Combe B, Cantagrel A, Goupille P, Olive P, Schattenkirchner M et al. Combination therapy in early rheumatoid arthritis: a randomised, controlled, double blind 52 week clinical trial of sulphasalazine and methotrexate compared with the single components. Ann Rheum Dis 1999; 58(4):220-225.
34. Haagsma CJ, van Riel PL, de Jong AJ, van de Putte LB. Combination of sulphasalazine and methotrexate versus the single components in early rheumatoid arthritis: a randomized, controlled, double-blind, 52 week clinical trial. Br J Rheumatol 1997; 36(10):1082-1088.

Chapter 5

Comparison of Treatment Strategies in Early Rheumatoid Arthritis

Y. P. M. Goekoop-Ruiterman
J. K. de Vries-Bouwstra
C. F. Allaart
D. van Zeben
P. J. S. M. Kerstens
J. M. W. Hazes
A. H. Zwinderman
A. J. Peeters
J. M. de Jonge-Bok
C. Mallée
W. M. de Beus
P. B. J. de Sonnaville
J. A. P. M. Ewals
F. C. Breedveld
B. A. C. Dijkmans

Ann Intern Med 2007; 146 (6): 406-15.

ABSTRACT

Background In patients with early rheumatoid arthritis, initial combination therapies provide earlier clinical improvement and less progression of joint damage after 1 year compared with initial monotherapies (as demonstrated in the BeSt study).

Objective To evaluate whether the initial clinical and radiographic efficacy of combination therapies could be maintained during the second year of follow-up in patients with early rheumatoid arthritis.

Design Randomized, controlled clinical trial with blinded assessors.

Setting 18 peripheral and 2 university medical centers in The Netherlands.

Patients 508 patients with early active rheumatoid arthritis.

Intervention Sequential monotherapy (group 1), step-up combination therapy (group 2), initial combination therapy with tapered high-dose prednisone (group 3), or initial combination therapy with infliximab (group 4). Trimonthly treatment adjustments were made to achieve low disease activity.

Measurements Primary end points were functional ability (Health Assessment Questionnaire) and Sharp-van der Heijde Score for radiographic joint damage.

Results Groups 3 and 4 had more rapid clinical improvement during the first year; all groups improved further to a mean functional ability score of 0.6 (overall, $P = 0.257$) and 42% were in remission (overall, $P = 0.690$) at the end of the second year. Progression of joint damage remained better suppressed in groups 3 and 4 (median scores of 2.0, 2.0, 1.0, and 1.0 in groups 1, 2, 3, and 4, respectively [$P = 0.004$]). After 2 years, 33%, 31%, 36%, and 53% in groups 1 through 4, respectively, were receiving single-drug therapy for initial treatment. There were no significant differences in toxicity.

Limitations Patients and physicians were aware of the allocated group, and the assessors were blinded.

Conclusions Currently available antirheumatic drugs can be highly effective in patients with early rheumatoid arthritis in a setting of tight disease control. Initial combination therapies seem to provide earlier clinical improvement and less progression of joint damage, but all treatment strategies eventually showed similar clinical improvements. In addition, combination therapy can be withdrawn successfully and less treatment adjustments are needed than with initial monotherapies.

INTRODUCTION

Over the last few years, the outcome for patients with rheumatoid arthritis has improved considerably (1). Combinations of disease modifying antirheumatic drugs (DMARDs) with corticosteroids and DMARDs with the new tumor necrosis factor-α antagonists seem to suppress the inflammatory process more effectively than single-drug therapy in patients with early (2-11) and established (12-15) disease. This results in less progression of radiographic joint damage and better preservation of physical function compared with single-drug therapy. More recently, tight control of disease activity has been shown to improve outcome (16), and there are indications that outcome is related to adherence to treatment guidelines (17). Whether combination therapy with DMARDs, corticosteroids, or tumor necrosis factor-α antagonists should be considered for initial treatment in all patients with rheumatoid arthritis or whether such therapy should be reserved for patients who do not respond to monotherapy is unknown. Therefore, the BeSt study (8) evaluated the efficacy of 4 of the most frequently used treatment strategies in a head-to-head comparison: sequential monotherapy (group 1), step-up combination therapy (group 2), initial combination therapy with tapered high-dose prednisone (group 3), and initial combination therapy with infliximab (group 4). Treatment adjustments were made every 3 months in patients with an insufficient response or a continued good response. During the first year of treatment, initial combination therapy for groups 3 and 4 resulted in more rapid clinical improvement and less progression of joint damage compared with initial monotherapy for groups 1 and 2. In the second year, response to therapy continued to be tightly monitored. Treatment adjustments were made according to the protocol. We evaluated whether the initial clinical and radiographic outcomes could be maintained and what treatment adjustments were needed in each group.

METHODS

Patients

Rheumatologists participating in the Foundation for Applied Rheumatology Research in 18 peripheral and 2 university hospitals in the western part of the Netherlands designed and conducted the BeSt study. Between April 2000 and August 2002, we recruited patients with early rheumatoid arthritis who fulfilled the American College of Rheumatology (ACR) 1987 criteria for rheumatoid arthritis (18). Patients were 18 years of age or older; had a disease duration of 2 years of less; had active disease with at least 6 of 66 swollen joints and at least 6 of 68 tender joints; and had an erythrocyte sedimentation rate of 28 mm/hr or greater or a global health score of 20 mm or more (on a visual analog scale of 0 mm [best] to 100 mm [worst]). Patient enrollment criteria have been described in detail previously (8). The Medical Ethics Committee at each participating center approved the study protocol, and all patients gave written informed consent before inclusion.

Treatment protocol

Patients were allocated to 1 of 4 treatment groups by variable block randomization (9-13), which was stratified per center. Treatment groups were sequential monotherapy (group 1), step-up combination therapy (group 2), initial combination therapy with tapered high-dose prednisone (group 3), and initial combination therapy with infliximab (group 4). For patients who did not respond to medication, the protocol prescribed many subsequent steps. The decision of whether to adjust medication was made every 3 months on the basis of the disease activity score (19), a continuous measure consisting of the Ritchie articular index and number of swollen joints in a 44-joint count, erythrocyte sedimentation rate, and global health as measured on a visual analog scale. A disease activity score of 2.4 or less indicates low disease activity (20). A research nurse who remained blinded for the allocated treatment group during the study period calculated the score. If the disease activity score was greater than 2.4 (insufficient response), the treating physician immediately adjusted therapy by proceeding to the next step in the allocated treatment group. If the disease activity score was 2.4 or less for at least 6 months, medication was gradually withdrawn until 1 drug remained in a maintenance dose.

Patients in group 1 started with methotrexate, followed subsequentially by sulphasalazine, leflunomide, methotrexate with infliximab, gold with methylprednisolone (intramuscular), methotrexate with cyclosporine A and prednisone, and azathioprine with prednisone. Patients in group 2 started with methotrexate, followed subsequently by methotrexate with sulphasalazine, methotrexate with sulphasalazine and hydroxychloroquine, methotrexate with sulphasalazine, hydroxychloroquine and prednisone, methotrexate with infliximab, methotrexate with cyclosporine A and prednisone, leflunomide, and azathioprine with prednisone. Patients in group 3 started with the combination of methotrexate, sulphasalazine, and tapered high-dose prednisone (2), followed subsequently by methotrexate with cyclosporine A and prednisone, methotrexate with infliximab, leflunomide, gold with methylprednisolone, and azathioprine with prednisone. Patients in group 4 started with the combination of methotrexate and infliximab, followed subsequently by sulphasalazine, leflunomide, methotrexate with cyclosporine A and prednisone, gold with methylprednisolone, and azathioprine with prednisone. In addition to the regular trimonthly assessments, we calculated additional disease activity scores every 8 weeks in all patients who were treated with infliximab in the week before infusion. On the basis of these scores, we decided whether to increase or taper the dosage. The treatment protocol and dose regimen have been described in detail previously (8).

We permitted concomitant treatment with nonsteroidal antiinflammatory drugs and intraarticular injections with corticosteroids and did not allow other parenteral corticosteroids. We allowed DMARDs or oral corticosteroids only as dictated by the treatment protocol. All patients received folic acid, 1 mg per day, during treatment with methotrexate.

Study end points and assessments

The primary efficacy end point was functional ability, as measured by the Dutch Health Assessment Questionnaire. Higher scores indicated more severe loss of physical function (21). Secondary efficacy end points were 20% and 70% improvement according to the ACR (ACR20 and ACR70, respectively) response criteria (22) and clinical remission, defined as a disease activity score less than 1.6 (23). Assessments were done every 3 months by blinded research nurses, who were trained at study onset and every 6 months thereafter to maintain consistency. Two study physicians ensured adherence to the protocol every 3 months. All protocol deviations were recorded. Patients were not informed about study outcomes until the end of the second year of follow-up.

The primary radiographic end point was the change in the total Sharp-van der Heijde score for joint damage, which ranged from 0 to 448, over 2 years (24). Two trained readers independently scored the radiographs of hands, wrists and feet at baseline and at the 2-year follow-up. The patient's identity, treatment group, and sequence of the films in sets were masked to the readers (JV and YG). We used the mean score of the 2 readers for the analysis. The intra-observer coefficients were 0.90 and 0.91, and the interobserver coefficient was 0.94. Erosive disease was defined as a mean erosion score greater than 0.5.

Toxicity

We performed physical examination and laboratory tests and recorded all adverse events at all visits. If necessary, the treating physician adjusted the patient's medication as outlined previously. Serious adverse events were defined as an adverse reaction resulting in any of the following outcomes: a life-threatening condition or death, substantial or permanent disability, malignant disease, hospitalization or prolongation of hospitalization, or a congenital abnormality or birth defect.

Before beginning infliximab therapy, all patients were evaluated for tuberculosis with a purified protein derivative skin test and chest radiography. In the beginning of 2002, congestive heart failure was added as a contraindication for treatment with infliximab. Previously enrolled patients with heart failure - functional classes I, II, III, and IV concomitant congestive heart failure, as defined by the New York Heart Association - who had already received infliximab, continued this therapy, and were closely monitored (25). If these patients' conditions seemed to be worsening, treatment with infliximab was discontinued. Worsening was defined as every transition to a higher functional class.

Statistical analysis

We needed a sample size of 468 patients (117 per group) to ascertain 80% power to detect a difference of at least 0.2 in the Health Assessment Questionnaire score. This was set as a clinically relevant difference with a 5% significance level and adjustment for multiple comparisons between groups, assuming a SD of 0.45. The sample size also ensured

greater than 80% power to detect a difference of 20% or greater in the change in score for radiographic joint damage. We performed intention-to-treat analyses. When appropriate, we analyzed outcomes with a oneway analysis of variance (post hoc, least significant difference test for primary outcomes and Tukey's test for secondary outcomes to correct for multiple comparisons), Kruskal-Wallis test (post hoc Mann-Whitney U test), and chi-square test.

We performed longitudinal data analysis of the primary outcomes with linear mixed-effects models with therapy group, time, and their interaction as fixed effects and center as a random effect. We did not make parametric assumptions regarding the change in pattern over time. Because all measurements were done at fixed times, we considered, in addition to the center as the random effect, different association models for the covariance structure between the repeated measures of the primary outcomes and used the structure with the lowest Akaike information criterion value. We found that the Health Assessment Questionnaire (HAQ) score was a first-order, autoregressive model with heterogeneous variances and that the Sharp-van der Heijde score for radiographic joint damage was an unstructured covariance matrix. We investigated the baseline variables of sex, age, body mass index, rheumatoid factor positivity, disease activity score, HAQ score, and total Sharp-van der Heijde score for radiographic joint damage separately as fixed covariates for potential effect modifiers and confounders potentials and their interactions with time and therapy group in the mixed-effects model.

Role of the funding sources

This study was funded by a grant from the Dutch College of Health Insurances (College voor zorgverzekeringen). Schering-Plough, B.V. and Centocor, Inc. provided additional funding and supplied the medication for patients in group 4. The funding sources had no role in study design; collection, analyses, and interpretation of all data; writing the article; and the decision to submit the manuscript for publication.

RESULTS

We randomly assigned a total of 508 patients to receive sequential monotherapy (group 1, $n = 126$), step-up combination therapy (group 2, $n = 121$), initial combination therapy with prednisone (group 3, $n = 133$), or initial combination therapy with infliximab (group 4, $n = 128$) (Figure 1). At baseline, the groups were balanced with respect to the demographic and disease characteristics (Table 1). Enrolled patients had a median disease duration of 23 weeks (interquartile range, 14 to 53 weeks) and had active disease with mean disease activity and HAQ scores of 4.4 (SD, 0.9) and 1.4 (SD, 0.7), respectively. Seventy-two percent of patients had joint erosions at baseline. Over time, 27 patients who were equally distributed across the treatment groups ($P = 0.474$) were lost to follow-up: 12 withdrew consent (7 declined follow-up, 4 discontinued all medications despite having no adverse events, and 1 moved from the area), 7 had a revised diagnosis, 1 discontinued treatment because of an adverse event, 4 died, and 3 were lost to follow-up

for other reasons (2 were admitted to a nursing home and 1 wanted to become pregnant) (Figure 1). Furthermore, 12 (10%), 11 (9%), 14 (11%), and 6 (5%) patients in groups 1, 2, 3, and 4, respectively ($P = 0.343$), did not adhere to the treatment protocol but were included in the intention-to-treat analysis.

Table 1. Baseline Characteristics *

	Sequential monotherapy (Group 1)	Step-up combination therapy (Group 2)	Initial combination with prednisone (Group 3)	Initial combination with infliximab (Group 4)
Mean age (SD), y	54 (13)	54 (13)	55 (14)	54 (14)
Women, n (%)	86 (68)	87 (72)	88 (66)	85 (66)
Median time from diagnosis (IQR), wk	2 (1-5)	2 (1-4)	2 (1-4)	3 (1-5)
Median symptom duration (IQR), wk	23 (14-54)	26 (14-56)	23 (15-53)	23 (13-46)
RF positive – n (%)	84 (67)	77 (64)	86 (65)	82 (64)
Mean DAS (SD)	4.5 (0.9)	4.5 (0.8)	4.4 (0.9)	4.3 (0.9)
Mean HAQ score (SD)	1.4 (0.7)	1.4 (0.6)	1.4 (0.7)	1.4 (0.7)
Total Sharp-van der Heijde score				
Mean (SD)	7.3 (9.5)	6.3 (6.9)	5.9 (6.5)	7.0 (10.0)
Median (IQR)	3.5 (1.5-9.5)	5.0 (1.5-8.1)	3.5 (1.5-8.5)	4.0 (1.5-8.5)
Erosion score				
Mean (SD)	4.1 (6.2)	3.5 (4.3)	3.3 (4.3)	3.9 (5.8)
Median (IQR)	2.0 (0.5-4.5)	2.0 (0.5-4.5)	2.0 (0.5-4.5)	2.0 (0.5-5.0)
Narrowing score				
Mean (SD)	3.2 (4.9)	2.8 (3.2)	2.6 (3.2)	3.1 (5.2)
Median (IQR)	1.0 (0.0-4.0)	2.0 (0.0-4.5)	1.5 (0.0-4.0)	1.5 (0.0-3.5)
Erosive disease, n (%)	89 (72)	82 (70)	93 (71)	93 (73)

*Baseline radiographs were available for 123, 118, 131, and 127 patients in groups 1, 2, 3, and 4, respectively. There were 126, 121, 133, and 128 patients in groups 1 to 4, respectively. IQR = interquartile range. RF = rheumatoid factor. DAS = Disease Activity score. HAQ = health Assesment Questionnaire

Therapy

Seventy-nine percent of patients in all groups achieved the predefined goal of low disease activity (disease activity score ≤ 2.4) (overall, $P = 0.554$). To accomplish this goal, more patients in groups 1 and 2 required treatment adjustments than in groups 3 and 4 (67%, 69%, 42%, and 28%, respectively) (Figure 2). Most patients in groups 3 and 4 who responded well to the initial combination of drugs were eventually maintained on only 1 drug. Thus, at the 2-year follow-up, 33%, 31%, 36%, and 53% of patients in groups 1 through 4, respectively, were taking a single drug as initial treatment. At the same time, 27%, 7%, 13%, and 18% of patients, respectively, received the combination of methotrexate and infliximab, which could be given to patients who were not responding to their medication no earlier than 12 months in group 1, 15 months in group 2, and 9 months in group 3, as dictated by the treatment protocol.

Clinical efficacy

During the first year of treatment, patients in groups 3 and 4 regained physical function, as measured by the HAQ, substantially earlier than patients in groups 1 and 2 (9). During the second year, physical function improved further in all groups, resulting in a 2-year change in scores of 0.7, 0.8, 0.9, and 0.9 in groups 1 through 4, respectively (mean overall change, 0.6, [$P = 0.257$]) (Table 2). The percentage of patients in clinical remission increased from 31% after the first year to 42% after the second year (overall at 2 years, $P = 0.690$) (Figure 3). A total of 22%, 21%, 28%, and 40% of patients in groups 1 through 4, respectively, achieved a continued low disease activity score (≤ 2.4 from 6 to 24 months).

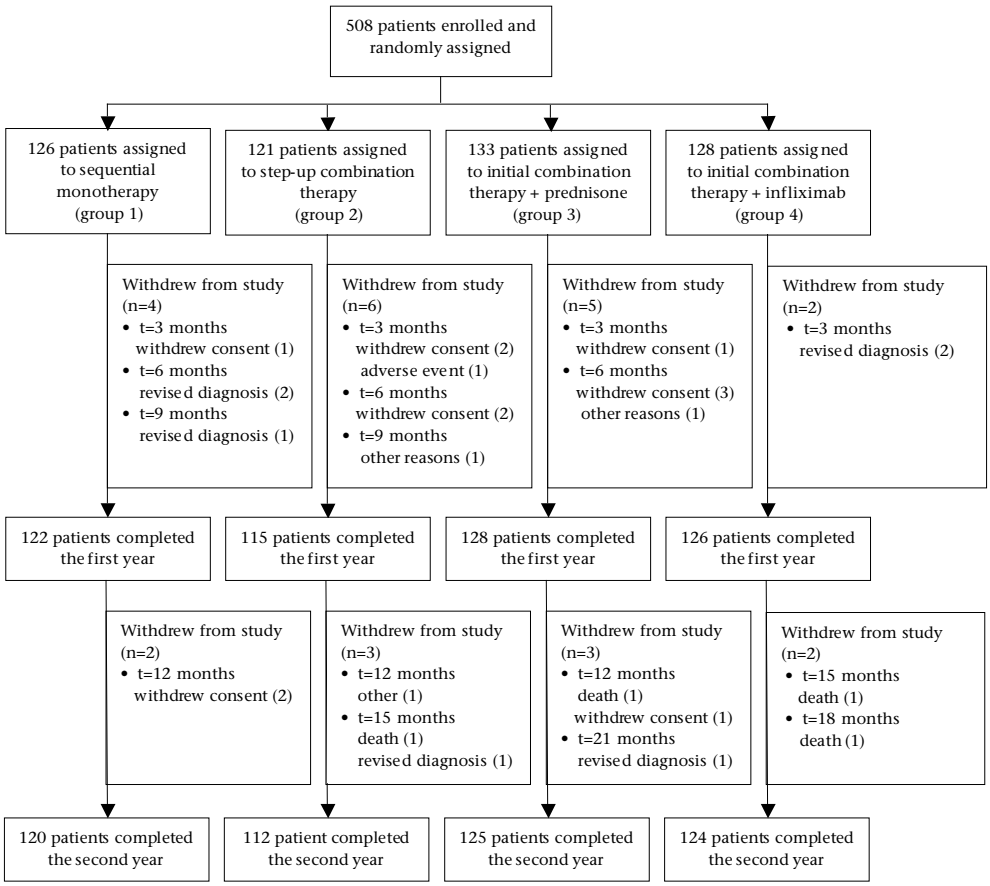


Figure 1. Study flow diagram
Revised diagnoses in the first year were paraneoplastic arthritis, gout, systemic lupus erythematosus, mixed connective tissue disease, Henoch-Schönlein purpura. Revised diagnoses in the second year were gout and sclerodermia. Causes of death were cerebrovascular accident (group 2), ovarian cancer (group 3), myocardial infarction (group 4), and disseminated tuberculosis (group 4).

Table 2. Primary patient outcomes during 2 years of follow-up*

	Sequential monotherapy (Group1)	Step-up combination therapy (Group2)	Initial combination with prednisone (Group3)	Initial combination with infliximab (Group4)	*P* Value
Mean improvement in health assessment questionnaire compared with baseline (SD)					
3 months	0.4 (0.6)	0.3 (0.6)	0.8 (0.7)	0.7 (0.6)	<0.001†
6 months	0.5 (0.7)	0.5 (0.7)	0.9 (0.7)	0.8 (0.6)	<0.001†
9 months	0.6 (0.7)	0.6 (0.7)	0.8 (0.7)	0.8 (0.6)	0.010†
12 months	0.7 (0.7)	0.7 (0.7)	0.9 (0.7)	0.9 (0.7)	0.031‡
15 months	0.7 (0.7)	0.8 (0.7)	0.7 (0.8)	0.9 (0.7)	0.299
18 months	0.7 (0.7)	0.8 (0.7)	0.8 (0.8)	0.9 (0.7)	0.255
21 months	0.7 (0.7)	0.8 (0.7)	0.8 (0.7)	0.9 (0.7)	0.220
24 months	0.7 (0.7)	0.8 (0.7)	0.9 (0.7)	0.9 (0.7)	0.257
Progression of Sharp-van der Heijde score compared with baseline					
Total score					
Mean (SD)	9.0 (17.9)	5.2 (8.1)	2.6 (4.5)	2.5 (4.6)	
Median (IQR)	2.0 (0.0-8.6)	2.0 (0.3-7.0)	1.0 (0.0-2.5)	1.0 (0.0-3.0)	0.005†
Erosion score					
Mean (SD)	4.7 (9.0)	3.1 (5.0)	1.1 (2.2)	1.3 (2.7)	
Median (IQR)	1.5 (0.0-5.6)	1.0 (0.0-5.3)	0.5 (0.0-2.0)	0.5 (0.0-2.0)	<0.001†
Narrowing score					
Mean (SD)	4.3 (9.8)	2.1 (3.8)	1.5 (3.2)	1.2 (2.9)	
Median (IQR)	0.0 (0.0-3.5)	0.5 (0.0-3.0)	0.0 (0.0-1.5)	0.0 (0.0-1.5)	0.072

*IQR = interquartile range; † $P < 0.050$ for all comparisons between groups 1 and 2 versus groups 3 and 4; ‡ $P < 0.050$ for group 1 versus groups 3 and 4.

Radiographic efficacy

Radiographs of the hands, wrists, and feet at baseline and at 2 year follow-up of 455 patients were available for analysis (111 [88%] in group 1, 105 [87%] in group 2, 123 [92%] in group 3, and 116 [91%] in group 4). The treatment groups had similar baseline radiographic joint damage (Table 1).

The patients in groups 3 and 4 had less progression of radiographic joint damage than that of those in groups 1 and 2. The median increase in total Sharp-van der Heijde score was 2.0, 2.0, 1.0, and 1.0 in groups 1 through 4, respectively (group 1 vs. 2, $P = 0.850$; group 1 vs. 3, $P = 0.043$; group 1 vs. 4, $P = 0.014$; group 2 vs. 3, $P = 0.006$; group 2 vs. group 4, $P = 0.004$; group 3 vs. 4, $P = 0.798$) (Table 2). Fewer patients in groups 3 and 4 had severe progression of the total Sharp-van der Heijde score compared with those in groups 1 and 2. An increase in total Sharp-van der Heijde score of more than 20 points in 2 years was seen in 18 patients, 7 patients, 1 patient, and 1 patient in groups 1 through 4 respectively (Figure 4).

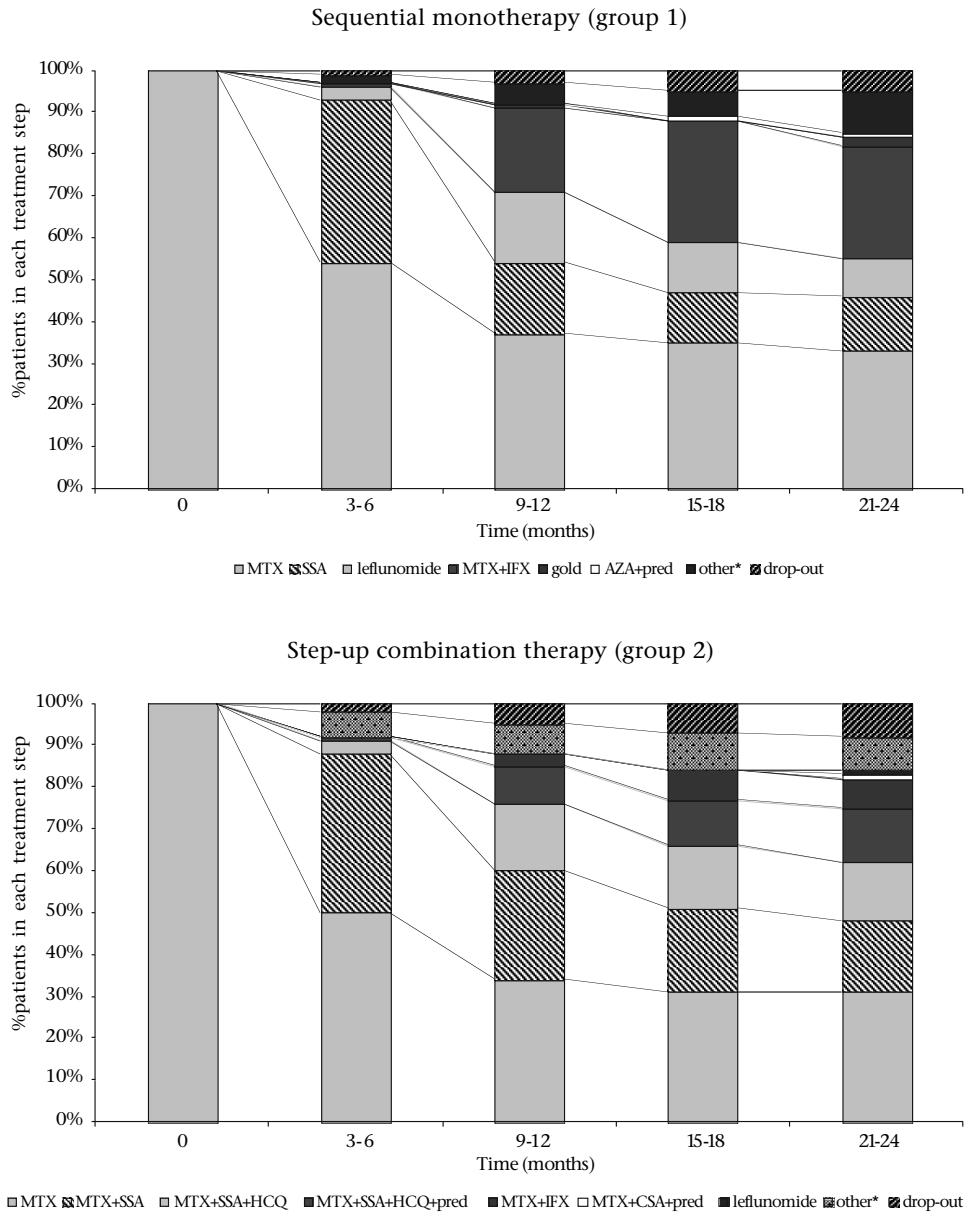
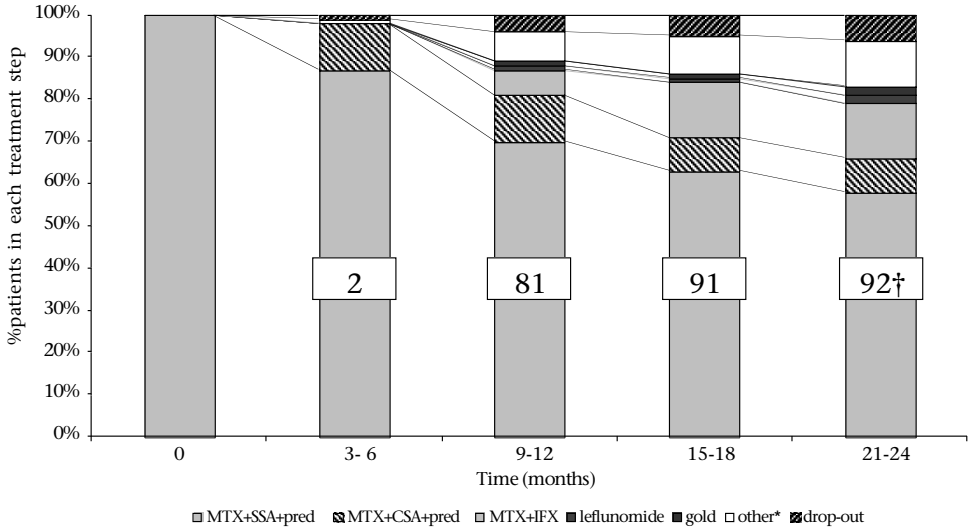
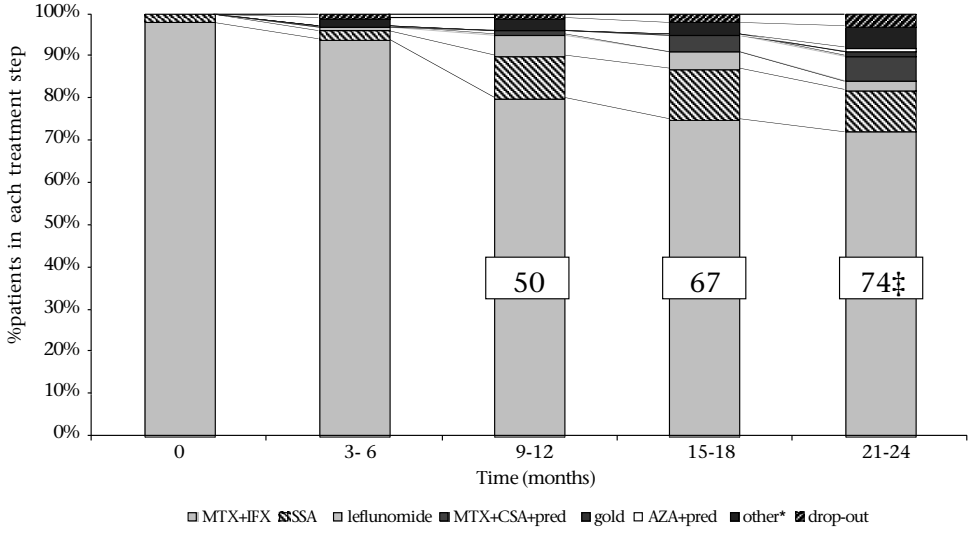


Figure 2. Treatment of patients during the second year of follow-up
*Percentage of patients that lost adherence to the treatment protocol. †Percentage of patients in the initial combination treatment group who discontinued prednisone (pred), because of a sustained disease activity score ≤ 2.4

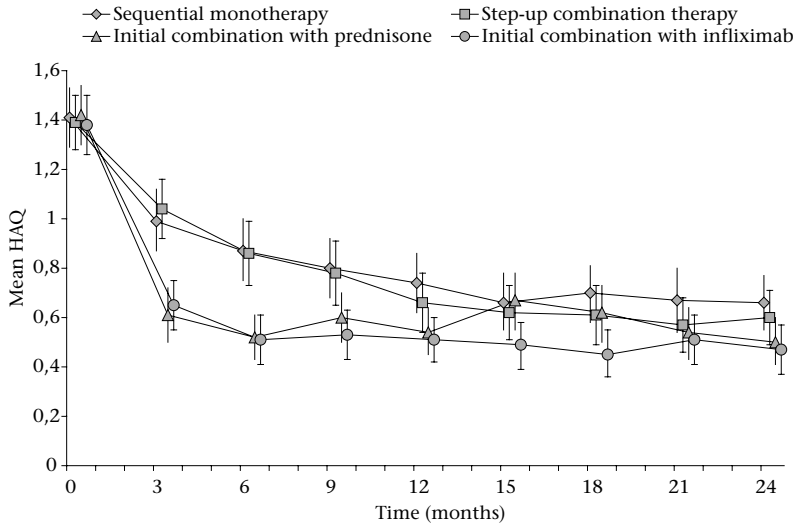
Initial combination with prednisone (group 3)

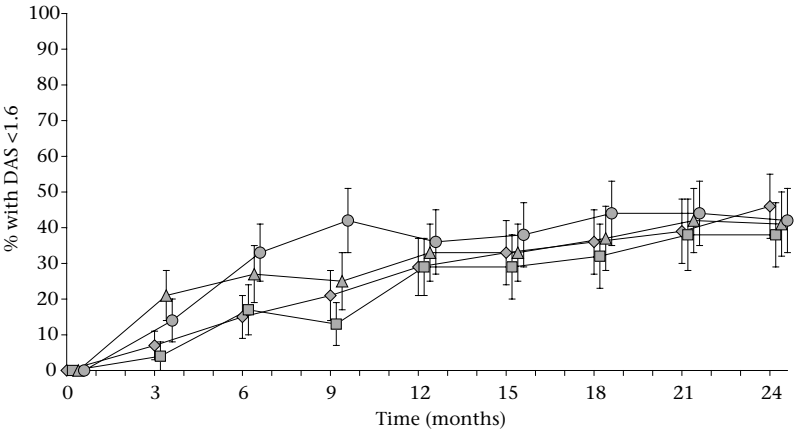


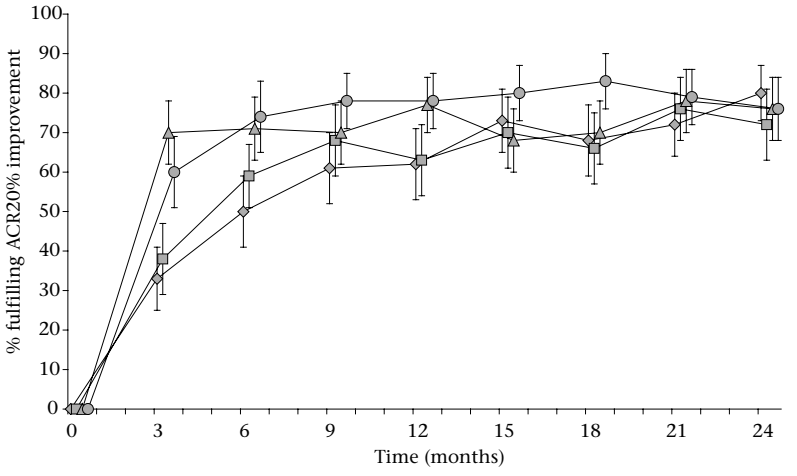
Initial combination with infliximab (group 4)



‡Percentage of patients in the initial combination treatment group who discontinued infliximab (IFX), because of a sustained disease activity score ≤ 2 .4.
MTX = methotrexate, SSA = sulphasalazine, HCQ = hydroxychloroquine, CSA = cyclosporine A, AZA = azathioprine.


Sequential monotherapy
Step-up combination therapy
Initial combination with prednisone
Initial combination with infliximab
Mean HAQ
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0
3
6
9
12
15
18
21
24
Time (months)


% with DAS <1.6
100
90
80
70
60
50
40
30
20
10
0
0
3
6
9
12
15
18
21
24
Time (months)


% fulfilling ACR20% improvement
100
90
80
70
60
50
40
30
20
10
0
0
3
6
9
12
15
18
21
24
Time (months)

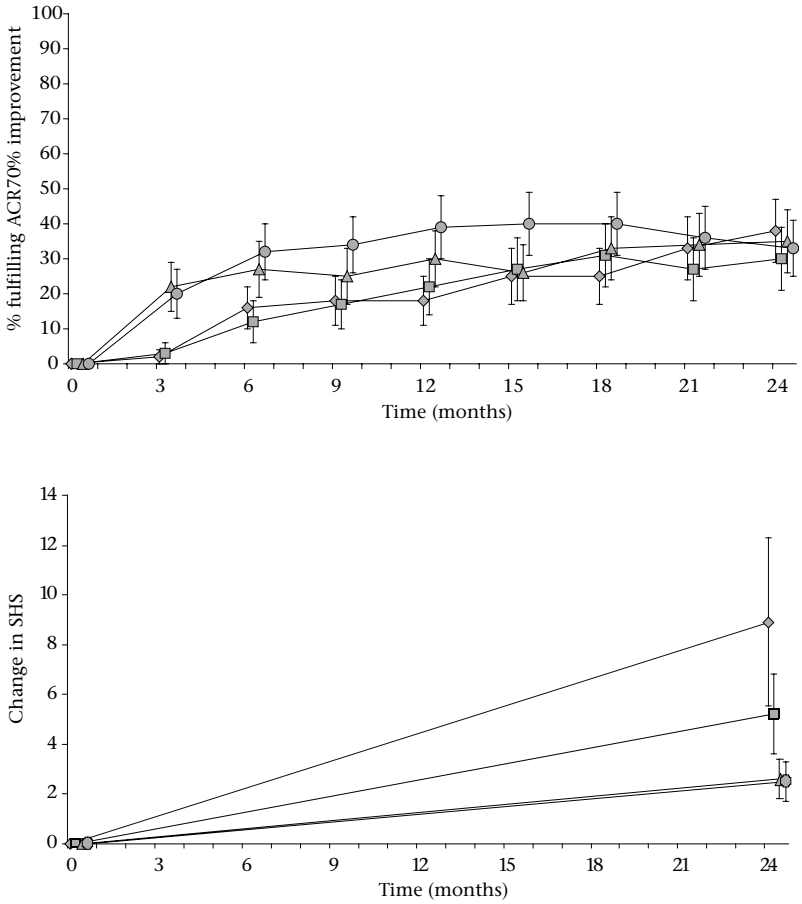


Figure 3. Clinical and radiographic efficacy outcomes during 2 years of follow-up
The error-bars indicate the 95% confidence intervals. HAQ = Health Assessment Questionnaire. A disease activity score (DAS) < 1.6 indicated clinical remission; ACR20 and ACR70 indicated 20% and 70% improvement, respectively, according to the American College of Rheumatology (ACR) criteria. SHS = Sharp-van der Heijde score for radiographic joint damage.

Mixed-model analysis

In the mixed-model analysis, groups 3 and 4 had significantly better HAQ scores over time than those of groups 1 and 2, and group 4 had better scores than those of group 3 (all pairwise comparisons between groups 1 or 2 and groups 3 or 4, $P < 0.001$; group 3 vs. group 4, $P = 0.021$; and group 1 vs. group 2, $P = 0.573$). Groups 3 and 4 also had less progression of the Sharp-van der Heijde score for radiographic joint damage over time than groups 1 and 2, and group 2 had less progression than group 1 (group 1 vs. group 2, $P = 0.044$; group 1 vs. groups 3 and 4, $P < 0.001$; group 2 vs. group 3, $P = 0.008$; group 2 vs. group 4, $P = 0.009$; group 3 vs. group 4, $P = 0.734$). We found an association among HAQ scores, sex, baseline disease activity score, and body mass index and an association between the Sharp-van der Heijde scores and age. None of these potential effect-modifying variables significantly changed outcomes.

Adverse events

Overall, 210 (41%) patients and 193 (38%) patients had at least 1 adverse event in the first and second year, respectively. The mean number of adverse events per patient was 1.9 (SD, 1.2) in the first year and 1.8 (SD, 1.2) in the second year. Most adverse events were classified as mild to moderate and lead to discontinuation or dose reduction of antirheumatic drugs in less than 11% of patients. Gastrointestinal adverse events were the most frequently reported events during the 2 years of follow-up . During the second year of follow-up, 14 (12%), 11 (9%), 12 (9%), and 15 (12%) patients in groups 1 through 4, respectively, had mild to moderate gastrointestinal events, including elevated liver enzyme levels. At least 5% of patients of groups 1 through 4 had other mild adverse events, including skin rash or other mild dermal or mucosal events in 12 (10%), 10 (8%), 15 (11%), and 7 (6%) patients, respectively; infections in 10 (8%), 10 (8%), 10 (8%), and 13 (10%) patients, respectively; and cardiovascular events 5 (4%), 5 (4%), 9 (7%), and 8 (6%) patients, respectively. In the second year, we observed 3 reactions to infliximab infusions (1 in group 1 and 2 in group 3). One infusion reaction in group 3 resulted in the patient being hospitalized overnight for observation of mild dyspnea and facial redness.

Serious adverse events were reported in 8, 9, 17, and 6 patients in groups 1 through 4, respectively, during the first year. A detailed description of these events has been published previously (8). During the second year, 56 serious adverse events were reported (16 events [13 patients] in group 1, 10 events [10 patients] in group 2, 17 events [11 patients] in group 3, and 13 events [8 patients] in group 4). In group 1, serious adverse events included (hospitalization for) atrial fibrillation, myocardial infarction, coronary artery bypass surgery, syncope, pyelonephritis, viral infection, perforated gastric ulcer, pleural effusion, ovarian cyst, methotrexate intoxication, malaise, and depressive symptoms in 1 patient each, and pneumonia in 1 patient, *Legionella* pneumonia in 1 patient, and malignant disease — basal-cell carcinoma and renal-cell carcinoma — in 1 patient each. In group 2, serious adverse events included

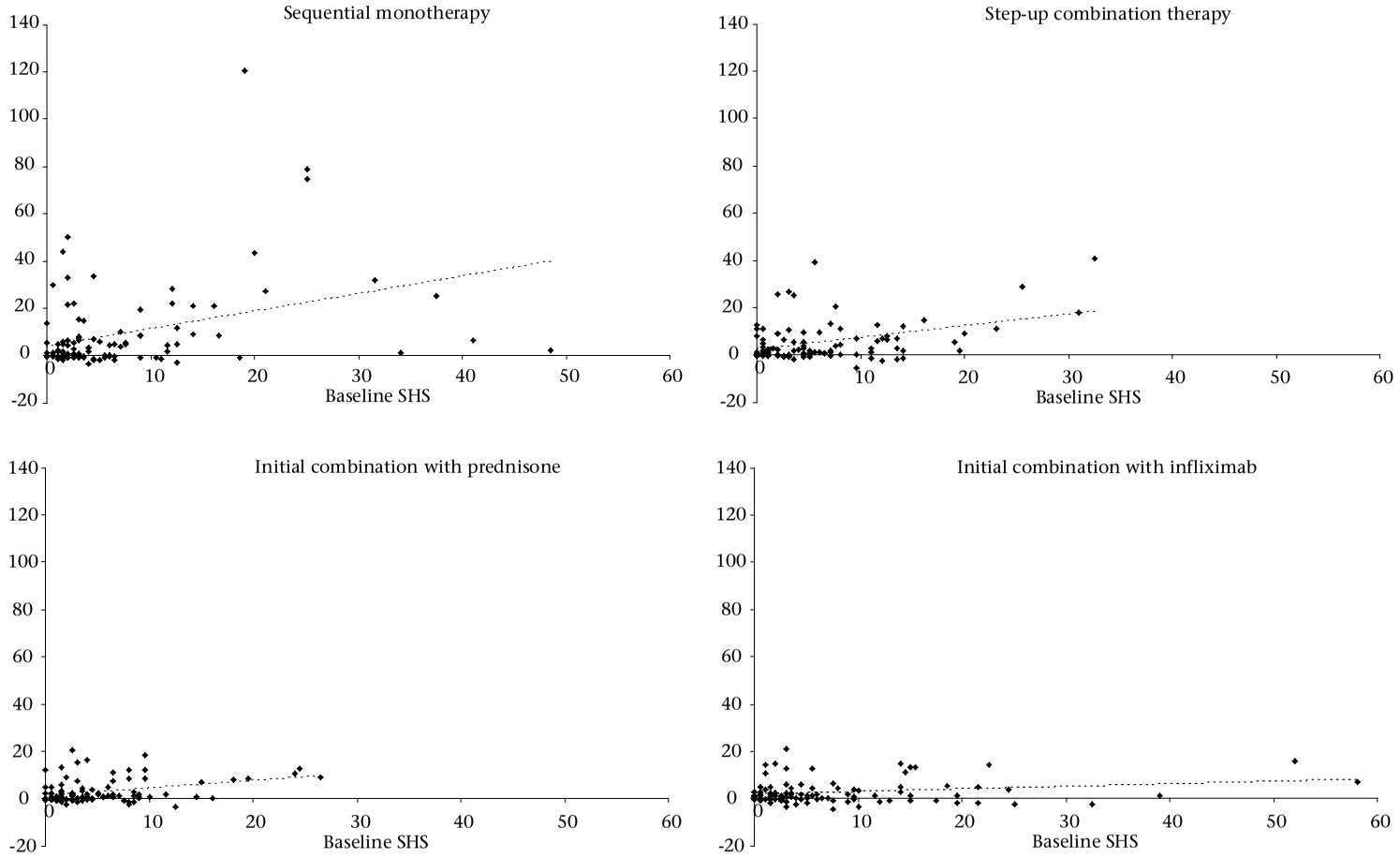


Figure 4. Median change in SHS (y-ax) by baseline total Sharp-van der Heijde score (SHS) for the 4 treatment groups
The dotted lines are regression lines.

(hospitalization for) pacemaker implantation, pneumonia, symptomatic gallstone disease, surgery for carpal tunnel syndrome, complicated calcaneal fracture, uterus extirpation, and malignant prostate cancer in 1 patient each and placement of total hip prostheses in 2 patients. One patient in group 2 died because of a cerebrovascular event. In group 3, serious adverse events included (hospitalization for) implantation of intracardiac device, syncope due to aortic valve dysfunction, limb amputation due to occlusion of femoral artery, pyelonephritis, viral infection, oral infectious ulcer, interstitial lung disease with respiratory failure, dyspnea during infliximab infusion, retinal hemorrhage, scleroderma, active rheumatoid arthritis, and 1 malignant, ovarian cancer (which resulted in death) in 1 patient each and placement of total hip prostheses in 2 patients. One patient had 2 retinal detachments. In group 4, serious adverse events included (hospitalization for) myocardial infarction, unstable angina pectoris, septic arthritis, gastrointestinal bleeding, cholecystectomy, placement of total knee prosthesis, placement of elbow prosthesis, active rheumatoid arthritis, and basal-cell carcinoma in 1 patient each. One patient had 4 episodes of disseminated tuberculosis. In group 4, 1 patient died of myocardial infarction and 1 died of disseminated tuberculosis. The patient who died from tuberculosis had positive results on purified protein derivative skin test (12-mm nodule) and an adhesive intrapulmonary lesion on the baseline chest radiograph. Six months of isoniazid prophylaxis was given, as recommended by the local guidelines at that time. One year after discontinuation of isoniazid therapy, while receiving methotrexate, 25 mg/week, and infliximab, 10 mg/kg, every 8 weeks, the patient received a diagnosis of meningitis tuberculosa, pulmonary *Aspergillus* infection, and mucocutaneous herpes simplex virus 2 infection (26). After a complicated disease course and several readmissions to the hospital, the patient died of septicemia with diffuse coagulation disorder and liver failure 7 months later.

DISCUSSION

The BeSt study has shown that a remarkable improvement of clinical signs and symptoms in patients with recent-onset rheumatoid arthritis can be achieved by using currently available drugs when treatment adjustments are made systematically and according to disease activity measurements. In all 4 treatment strategies studied, 29% to 36% of patients achieved clinical remission (disease activity score < 1.6) after the first year of therapy, which increased to 38% to 46% after the second year. Seventy-nine percent of patients achieved the goal of low disease activity (disease activity score ≤ 2.4) after 2 years. In addition, functional ability improved and progression of joint damage was suppressed effectively with all treatment strategies. Compared with previous trials reporting a median Sharp-van der Heijde score progression of 3.2 to 12 over 1.5 to 2 years for patients with early rheumatoid arthritis who receive monotherapy (2,6,12), we observed the median progression of 2.0 in our groups with initial monotherapy (groups 1 and 2), suggesting effective suppression in these patients. Because of consequent monitoring and adjustment of medications, which

is more dynamic than in current daily practice, patients in groups 1 and 2 achieved almost the same improvement in disease activity and functional ability after 2 years as those who started with combination therapy (groups 3 and 4). Medication had to be adjusted more often in patients in groups 1 and 2 than in patients in groups 3 and 4 to achieve this improvement. As a result, even more patients in the initial combination groups were successfully treated with monotherapy than those who were treated with initial monotherapy after 2 years.

During the first year of treatment, patients in groups 3 and 4 achieved lower levels of disease activity than did those in groups 1 and 2. Patients in groups 3 and 4 also had less radiographic progression of joint damage after 2 years. In particular, severe progression was seen less often in groups 3 and 4 than in groups 1 and 2 despite the high baseline Sharp-van der Heijde score (median, 4) and erosion percentage (72%). The traditionally observed linear association between baseline damage and progression of damage over time was seen in group 1, was less acute in group 2, and was not seen in groups 3 and 4. One may argue the significance of the differences in disease outcomes between the initial combination therapy strategies and the strategies starting with initial monotherapy when tight disease control is applied. We conclude that the difference in disease activity during the first year is clinically relevant and will probably have an economic impact. Patients with active disease are known to discontinue working, and the success of reintegration into the workforce is inversely related to the duration of sick leave (27).

The clinical relevance of the difference in joint damage progression is less clear. The small difference observed has not translated into differences in functional capacity. In the years after the 2-year observation period, tight disease control in the patients with initial monotherapy may lead physicians to prescribe combination therapy. Further progression of joint damage, similar to that observed in patients who started treatment with combination therapy, would be suppressed. However, intense suppression of rheumatoid arthritis activity as early as possible may result in more mild disease and less joint destruction. Long-term follow-up of our patient groups with respect to functional and radiographic outcomes will provide more insight.

The therapeutic advantage of initial combination therapy is not counterbalanced with increased toxicity. Vigilance in recognizing and treating serious infections is necessary, as demonstrated by the case of 1 patient in our study. The risk of reactivation of tuberculosis in patients who start tumor necrosis factor-α blocking therapies warrants screening and treatment for tuberculosis according to local guidelines.

On the basis of these results, we recommend that physicians increase their targets regarding suppression of disease activity and prevention of joint damage in patients with recent-onset rheumatoid arthritis. With intensive and objective monitoring of disease activity and adjustments of therapy, low disease activity is a realistic goal that can be achieved with all treatment strategies. Initial combination treatment with tapered high-dose prednisone, methotrexate and sulphasalazine, or infliximab and methotrexate seems to be the best choice to rapidly achieve this goal in patients with active rheumatoid arthritis of recent onset.

Acknowledgments

The authors thank the following participants of the Foundation for Applied Rheumatology Research for their contribution to the design and the conduction of the study: M.H.W. de Bois MD, Medical Center Haaglanden, The Hague; G. Collée MD, Medical Center Haaglanden, The Hague; A. H. Gerards MD, Vlietland Hospital, Schiedam; B. A. M. Grillet MD, De Honte Hospital, Terneuzen; J. H. L. M. van Groenendael MD, Franciscus Hospital, Roosendaal; K. H. Han MD, Medical Center Rijnmond Zuid, Rotterdam; H. M. J. Hulsmans MD, Haga Hospital, The Hague; M. H. de Jager MD, Albert Schweitzer Hospital, Dordrecht; M. V. van Krugten MD, Walcheren Hospital, Vlissingen; H. van der Leeden (retired); W. F. Lems MD, Slotervaart Hospital, Amsterdam; M. F. van Lieshout-Zuidema MD, Spaarne Hospital, Hoofddorp; A. Linssen MD, Kennemer Gasthuis, Haarlem; P. A. H. M. van der Lubbe MD, Vlietland Hospital, Schiedam; H. K. Markusse (deceased); H. K. Ronday MD, Haga Hospital, The Hague; D. van Schaardenburg MD, VU Medical Center, Amsterdam and Jan van Breemen Institute, Amsterdam; P. E. H. Seys MD, Lievensberg Hospital, Bergen op Zoom; R. M. van Soesbergen (retired); I. Speyer MD, Bronovo Hospital, The Hague; J. P. Terwiel MD, Spaarne Hospital, Hoofddorp; A.E. Voskuyl MD, VU Medical Center, Amsterdam; M.L. Westedt MD, Bronovo Hospital, The Hague; S. ten Wolde MD, Kennemer Gasthuis, Haarlem.

Grant support

A government grant of the Dutch College of Health Insurance Companies was provided, with additional grants of Schering-Plough B.V. and Centocor, Inc. who also supplied infliximab study medication for patients in group 4.

REFERENCES

1. Pincus T, Sokka T, Kautiainen H. Patients seen for standard rheumatoid arthritis care have significantly better articular, radiographic, laboratory, and functional status in 2000 than in 1985. Arthritis Rheum 2005; 52(4):1009-1019.
2. Boers M, Verhoeven AC, Markusse HM, van de Laar MA, Westhovens R, van Denderen JC et al. Randomised comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis. Lancet 1997; 350(9074):309-318.
3. Breedveld FC, Emery P, Keystone E, Patel K, Furst DE, Kalden JR et al. Infliximab in active early rheumatoid arthritis. Ann Rheum Dis 2004; 63(2):149-155.
4. Breedveld FC, Weisman MH, Kavanaugh AF, Cohen SB, Pavelka K, van Vollenhoven R et al. The PREMIER study: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. Arthritis Rheum 2006; 54(1):26-37.
5. Calguneri M, Pay S, Caliskaner Z, Apras S, Kiraz S, Ertenli I et al. Combination therapy versus monotherapy for the treatment of patients with rheumatoid arthritis. Clin Exp Rheumatol 1999; 17(6):699-704.
6. Genovese MC, Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH et al. Etanercept versus methotrexate in patients with early rheumatoid arthritis - Two-year radiographic and clinical outcomes. Arthritis Rheum 2002; 46(6):1443-1450.
7. Gerards AH, Landewe RB, Prins AP, Bruyn GA, Goei The HS, Laan RF et al. Cyclosporin A monotherapy versus cyclosporin A and methotrexate combination therapy in patients with early rheumatoid arthritis: a double blind randomised placebo controlled trial. Ann Rheum Dis 2003; 62(4):291-296.
8. Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Allaart CF, van Zeben D, Kerstens PJ, Hazes JM et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): a randomized, controlled trial. Arthritis Rheum 2005; 52(11):3381-3390.
9. Klareskog L, van der HD, de Jager JP, Gough A, Kalden J, Malaise M et al. and the TEMPO (Trial of Etanercept and Methotrexate with Radiographic Patient Outcomes) study investigators. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. Lancet 2004; 363(9410):675-681.
10. Mottonen T, Hannonen P, Leirisalo-Repo M, Nissila M, Kautiainen H, Korpela M et al. and the FIN-RACo trial group. Comparison of combination therapy with single-drug therapy in early rheumatoid arthritis: a randomised trial. Lancet 1999; 353(9164):1568-1573.
11. St Clair EW, van der Heijde DM, Smolen JS, Maini RN, Bathon JM, Emery P et al. Combination of infliximab and methotrexate therapy for early rheumatoid arthritis: a randomized, controlled trial. Arthritis Rheum 2004; 50(11):3432-3443.

12. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Furst D, Weisman MH et al. Sustained improvement over two years in physical function, structural damage, and signs and symptoms among patients with rheumatoid arthritis treated with infliximab and methotrexate. Arthritis Rheum 2004; 50(4):1051-1065.
13. O'Dell JR, Haire CE, Erikson N, Drymalski W, Palmer W, Eckhoff PJ et al. Treatment of rheumatoid arthritis with methotrexate alone, sulfasalazine and hydroxychloroquine, or a combination of all three medications. N Engl J Med 1996; 334(20):1287-1291.
14. Tugwell P, Pincus T, Yocum D, Stein M, Gluck O, Kraag G et al. The Methotrexate-Cyclosporine Combination Study Group. Combination therapy with cyclosporine and methotrexate in severe rheumatoid arthritis. N Engl J Med 1995; 333(3):137-141.
15. Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI et al. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N Engl J Med 1999; 340(4):253-259.
16. Grigor C, Capell H, Stirling A, McMahon AD, Lock P, Vallance R et al. Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomised controlled trial. Lancet 2004; 364(9430):263-269.
17. Fransen J, Moens HB, Speyer I, van Riel PL. Effectiveness of systematic monitoring of rheumatoid arthritis disease activity in daily practice: a multicentre, cluster randomised controlled trial. Ann Rheum Dis 2005; 64(9):1294-1298.
18. Arnett FC, Edworthy SM, Bloch DA, Mcshane DJ, Fries JF, Cooper NS et al. The American-Rheumatism-Association 1987 revised criteria for the classification of rheumatoid arthritis 2. Arthritis Rheum 1988; 31(3):315-324.
19. van der Heijde DM, Van 't Hof M, van Riel PLCM, Van de Putte LBA. Development of a disease activity score based on judgment in clinical practice by rheumatologists. J Rheumatol 1993; 20(3):579-581.
20. Van Gestel AM, Prevoo MLL, VantHof MA, VanRijswijk MH, VandePutte LBA, vanRiel PLCM. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis - Comparison with the preliminary American College of Rheumatology and the World Health Organization International League Against Rheumatism criteria. Arthritis Rheum 1996; 39(1):34-40.
21. Siegert CEH, Vleming LJ, Vandenbroucke JP, Cats A. Measurement of Disability in Dutch Rheumatoid-Arthritis Patients. Clin Rheumatol 1984; 3(3):305-309.
22. Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C et al. American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995; 38(6):727-735.
23. Prevoo MLL, VanGestel AM, VantHof MA, VanRijswijk MH, VandePutte LBA, vanRiel PLCM. Remission in a prospective study of patients with rheumatoid arthritis. American Rheumatism Association preliminary remission criteria in relation to the disease activity score. Br J Rheumatol 1996; 35(11):1101-1105.
24. van der Heijde DM, van Riel PL, Gribnau FW, Nuverzwart IH, Vandeputte LB. Effects of hydroxychloroquine and sulphasalazine on progression of joint damage in rheumatoid arthritis. Lancet 1989; 1(8646):1036-1038.

25. The criteria committee of the New York Heart Association. Nomenclature and criteria for diagnosis. 9th ed. 1994. Boston: Little, Brown. Ref Type: Report
26. van der Klooster JM, Bosman RJ, Oudemans-van Straaten HM, van der Spoel JI, Wester JP, Zandstra DF. Disseminated tuberculosis, pulmonary aspergillosis and cutaneous herpes simplex infection in a patient with infliximab and methotrexate. Intensive Care Med 2003; 29(12):2327-2329.
27. Puolakka K, Kautiainen H, Mottonen T, Hannonen P, Korpela M, Julkunen H et al. Impact of initial aggressive drug treatment with a combination of disease-modifying antirheumatic drugs on the development of work disability in early rheumatoid arthritis: a five-year randomized followup trial. Arthritis Rheum 2004; 50(1):55-62.

Chapter 6

Initial combination therapy in early rheumatoid arthritis results in remission with less progression of joint damage than initial monotherapy

J. K. de Vries-Bouwstra
Y. P. M. Goekoop-Ruiterman
P. A. H. M. van der Lubbe
M. J. Westedt
D. van Schaardenburg
A. H. Zwinderman
F. C. Breedveld
J. M .W. Hazes
B. A. C. Dijkmans
C. F. Allaart

submitted

ABSTRACT

Objective To evaluate progression of joint damage and functional ability in patients with early rheumatoid arthritis (RA) with clinical remission and with clinical failure receiving initial monotherapy or initial combination therapy.

Methods The BeSt study was a randomized clinical trial comparing 4 targeted (DAS ≤ 2.4) treatment strategies in 508 early RA patients: (1) sequential monotherapy, (2) step-up therapy (both starting with methotrexate monotherapy), and initial combination therapy with (3) methotrexate + sulphasalazine + prednisone, or (4) methotrexate + infliximab. The numbers of patients with continuous (6-24 months) remission (DAS < 1.6; 1 x DAS ≥ 1.6 allowed) and with continuous failure (DAS > 2.4; 1 x DAS ≤ 2.4 allowed) were determined. Progression of joint damage (percentage with Sharp-van der Heijde score > smallest detectable change) and functional ability (area under the curve for Health Assessment Questionnaire [HAQ]) were compared between *remissions* and *failures* with initial monotherapy (groups 1 + 2) or initial combination therapy (groups 3 + 4).

Results Continuous remission was more frequent in patients receiving initial combination therapy (n = 40 / 232) than in patients receiving initial monotherapy (n = 21 / 249). *remissions* on combination therapy had significantly less often progression than *remissions* on monotherapy (3% vs 25%; $P < 0.01$). *Failures* on combination therapy had lower HAQ than *failures* on monotherapy (1.5 [1.0-1.8] vs 1.0 [0.8-1.5]; $P = 0.02$).

Conclusion More RA patients receiving initial combination therapy achieved continuous remission compared with patients receiving initial monotherapy. *Remissions* and *failures* both benefited from initial combination therapy; *remissions* by having less joint damage progression, *failures* by achieving better functional ability, compared with patients receiving initial monotherapy.

INTRODUCTION

Because a strong relation between persistent disease activity and joint damage progression has been demonstrated (1-3), early and sustained suppression of disease activity is a main goal in the treatment of rheumatoid arthritis (RA). This approach results in significantly better functional and radiographic outcome for patients with RA (4,5). However, it is unclear whether early suppression of disease activity with a single disease modifying antirheumatic drug (DMARD) is as effective in inhibiting progression of joint damage as is a combination of DMARDs.

Recent data suggest that infliximab treatment may help prevent the progression of joint damage in patients with RA (6,7). Data from the ATTRACT-study (6) showed that despite the lack of clinical response (ACR20), the combination of infliximab and methotrexate (MTX) resulted in lower joint damage progression than MTX alone in ACR20 nonresponders. This effect on the prevention of joint damage also appears to be demonstrated by other TNF-α blockers (7,8). Based on these observations it has been suggested that synovitis and destruction are two associated but separate components of the inflammatory process and that TNF-α blockade is particularly effective in inhibiting bone destruction.

Several studies have shown that treatment with prednisone is also effective in preventing joint damage progression (9), but whether this also represents a disconnection between the effect on disease activity and joint damage is not known.

In the BeSt study, which compared four treatment strategies in early RA: sequential monotherapy, step-up combination therapy (both starting with methotrexate monotherapy for 6 months), initial combination therapy including prednisone, and initial combination therapy with the TNF-α inhibitor infliximab, patients treated with initial combination therapy showed less joint damage progression than patients treated with initial monotherapy (10).

To investigate a possible disconnection between clinical and radiographic efficacy, we compared the radiographic outcomes of patients receiving initial monotherapy who achieved continuous clinical remission (hereafter called *remissions*) and of patients who continuously failed on treatment (hereafter called *failures*) with those of *remissions* and *failures* receiving initial combination therapy. In addition, functional outcomes were compared between *remissions* and *failures* receiving either initial monotherapy or initial combination therapy.

PATIENTS AND METHODS

Design

This study was performed using data from the BeSt study, a 2-year, multi-center, single-blinded, randomized clinical trial comparing the efficacy of four different treatment strategies in early RA. Detailed information with respect to methods, treatment, and primary outcomes is published elsewhere (10).

Patients

The 508 patients who participated in the BeSt trial had newly diagnosed RA (ACR 1987 criteria) and symptom duration of less than 2 years. For the current subanalysis, data from the 481 patients who completed the study were used.

Treatment protocol

Patients were randomly assigned to: group 1 (n = 126), sequential monotherapy, starting with MTX 15 mg/wk, group 2 (n = 121), step-up combination therapy, starting with MTX 15 mg/wk, group 3 (n = 133), initial combination therapy, starting with the combination of MTX 7.5 mg/wk, sulphasalazine 2000 mg/day, and a tapered high-dose prednisolone, and group 4 (n = 128) initial combination therapy starting with the combination of the TNF-α antagonist infliximab 3 mg/kg and MTX 25 mg/wk. The treatment goal was good clinical response as defined by the Disease Activity Score (DAS ≤ 2.4) in all four groups (11). The DAS was calculated every 3 months. In case of a DAS > 2.4, treatment was adjusted according to the pharmacoprotocol. In groups 1 and 2, if DAS > 2.4 on MTX 15 mg/wk, MTX was increased to 25 mg/wk. In group 1, MTX was subsequently replaced by sulphasalazine, leflunomide, and then by infliximab + MTX, in case of persistent failure. In group 2, subsequent sulphasalazine, hydroxychloroquine, and low dose prednisone were added to MTX, in case of persistent failure on MTX 25 mg/wk. Thereafter, if DAS > 2.4, infliximab + MTX were introduced. In group 3, if DAS > 2.4 on MTX (dose increased to 25 mg/wk) + sulphasalazine + low dose prednisone, subsequent treatments were combinations of MTX + cyclosporine + prednisone and MTX + infliximab. In group 4, if DAS > 2.4 on MTX 25 mg/wk + infliximab 3 mg/kg, first the dose of infliximab was increased up to 10 mg/kg/8 wks, and subsequently patients switched to sulphasalazine and then leflunomide. If the DAS was ≤ 2.4 for at least 6 months, the number and doses of drugs were tapered until only one DMARD remained in a maintenance dose. In groups 1 and 2, MTX was tapered to 10 mg/wk. In group 3, prednisone was tapered to 0, followed by MTX. In group 4, infliximab was tapered to 0 and then MTX was tapered to 10 mg/wk.

No statistically significant differences in the clinical and radiographic outcomes were found between both initial combination regimens. Also, the clinical and radiographic outcomes of groups 1 and 2 were very similar (10). Therefore, for the current analyses, groups 1 and 2 (initial monotherapy) were compared with groups 3 and 4 (initial combination therapy). In addition, progression of joint damage was compared between *remissions* and *failures* in group 3 and group 4.

Assessments

A research nurse who was blinded to the assigned treatment groups made assessments of the DAS and the Health Assessment Questionnaire (HAQ) every 3 months (12). Radiographs of patients' hands and feet were taken at baseline and after two years of follow-up. All radiographs were scored according to the Sharp-van der Heijde (SHS)

method, paired, in random order, by two independent observers who were blinded for clinical data (13). The mean score of the two independent observers was used for the analysis. A patient was defined to have erosive disease if the mean erosion score was > 0.5. Progression of joint damage was defined as a difference between 2 year and baseline > Smallest Detectable Change (SDC) determined at 4.64 points (10).

Analysis

Outcome parameters for the current analyses were progression of joint damage (SHS) over 2 years and cumulative functional ability (area under the curve [AUC] for HAQ) over 2 years.

To investigate a possible effect of combination therapy on the association between joint damage progression and functional ability, all patients who achieved continuous clinical remission and patients who failed on subsequent treatment steps were selected. *Remissions* were defined as all patients with continuous DAS < 1.6 between 6 and 24 months of follow-up (1 DAS out of 7 calculated between 6 and 24 months ≥ 1.6 but ≤ 2.4 was allowed) (14). *Failures* were defined as all patients with continuous DAS > 2.4 between 6 and 24 months of follow-up (1 DAS out of 7 calculated between 6 and 24 months ≤ 2.4 but ≥ 1.6 was allowed). The numbers of *remissions* and *failures* with initial monotherapy and initial combination therapy were determined. *Remissions* and *failures* were evaluated for progression of joint damage and for functional ability with initial monotherapy as compared with initial combination therapy. *Remissions* and *failures* were evaluated for progression of joint damage and for functional ability for initial combination therapy with prednisone as compared with initial combination therapy with infliximab. Differences in baseline characteristics and outcome parameters between *remissions* and *failures* were analyzed by the Chi-square, Student's t-test, or Mann Whitney-U where appropriate.

RESULTS

Baseline characteristics

Baseline characteristics for patients with initial monotherapy (groups 1 and 2) and for patients with initial combination therapy (groups 3 and 4) are shown in Table 1. At baseline, patient characteristics in both groups were comparable except for mean DAS, which was 0.2 points higher in the group with monotherapy. A detailed description of overall results of the BeSt study is published elsewhere (10). In summary, over 2 years initial combination therapy resulted in significantly lower HAQ and less joint damage progression as compared with initial monotherapy (Table 1). Mean DAS was comparable during the second year of follow-up, and due to the earlier suppression of disease activity the cumulative DAS over 2 years was significantly lower with initial combination therapy.

Table 1. Baseline characteristics and outcomes over 2 years of follow-up for patients with initial monotherapy (n = 232) and for patients with initial combination therapy (n = 249)

	Initial monotherapy (n = 232)	Initial combination therapy (n = 249)	*P* value
Baseline characteristics			
Age, mean (SD)	54 (13)	54 (14)	0.74
Females, no (%)	164 (71)	161 (65)	0.16
RF positive, no (%)	155 (67)	158 (64)	0.44
Duration of symptoms in weeks, mean (SD)	25 (14-55)	23 (14-51)	0.56
CRP, median (IQR) ∞	25 (9-59)	22 (10-55)	0.69
HAQ, median (IQR)	1.4 (1.0-1.9)	1.4 (0.9-1.8)	0.83
DAS, mean (SD)	4.5 (0.9)	4.3 (0.9)	0.02
SHS			
mean (SD)	6.6 (8.1)	6.4 (8.5)	
median (IQR)†	4.0 (1.5-8.5)	3.5 (1.5-8.5)	0.55
Erosive disease, number (%)†	163 (72)	175 (71)	0.87
Outcomes after 2 years of treatment			
Progression of joint damage, no (%)º	80 (37)	45 (19)	<0.01
SHS progression			
mean (SD)	7.1 (14.1)	2.6 (4.5)	
median (IQR)º	2.0 (0.0-8.0)	1.0 (0.0-3.0)	<0.01
HAQ, median (IQR) ‡	0.7 (0.4-1.1)	0.5 (0.2-0.8)	<0.01
DAS ≤ 2.4 on initial treatment step, no (%)	79 (34)	166 (67)	<0.01

∞ Groups 1+2 n = 209, Groups 3+4 n = 230; ◦ Groups 1+2 n = 196, Groups 3+4 n=207; † SHS = Sharp-Van der Heijde score; Groups 1+2 n = 227; Groups 3+4 n = 246; ‡Area under the curve for HAQ, Groups 1+2 n = 229; Groups 3+4 n = 247; ºGroups 1+2 n=215; Groups 3+4 n = 239. RF = rheumatoid factor, DAS = disease activity score, HAQ = Health Assesment Questionnaire, IQR = interquartile range

Remissions and failures

Out of the total number of 481 patients, 61 patients were classified as *remissions* and 43 patients were classified as *failures*. At baseline, *remissions* and *failures* did not differ from the other BeSt patients except for gender, mean DAS, median HAQ (Table 2), and patients' self reported scores for pain, morning stiffness, disease activity, and general wellbeing (all measured by Visual Analog Scale score; data not shown).

The number of *failures* among patients treated with initial monotherapy (n = 21; group 1 n = 12, group 2 n = 9) was comparable with the number of *failures* among patients treated with initial combination therapy (n = 22; group 3 n = 12, group 4 n = 10; *P* = 0.93 for initial monotherapy compared with initial combination therapy). At baseline there were no significant differences between *failures* on initial monotherapy and *failures* on initial combination therapy. More patients with initial combination therapy were classified as *remissions* (n = 40 group 3 n = 19, group 4 n = 21) than with initial monotherapy (n = 21; group 1 n = 15, group 2 n = 6; *P* = 0.02 for initial monotherapy compared with initial combination therapy). *Remissions* with initial combination therapy had a significantly

higher mean [SD] HAQ at baseline (1.2 [0.6]) as compared with *remissions* with initial monotherapy (0.9 [0.5]; $P = 0.036$]), otherwise baseline characteristics were comparable. Among *remissions* with initial combination therapy, 38% had achieved remission after 3 months of follow-up compared with 19% among *remissions* with initial monotherapy ($P = 0.14$).

Table 2. Baseline characteristics and outcomes over 2 years of follow-up for *remissions* and *failures*

	Remissions (n = 61)	Failures (n = 43)	*P* value
Baseline characteristics			
Age, mean (SD)	54 (14)	53 (14)	0.89
Females, no (%)	27(44)	37 (86)	<0.01
RF positive, no (%)	34 (56)	28(65)	0.34
Duration of symptoms in weeks, mean (SD)	24 (14-44)	23 (12-50)	0.59
CRP, median (IQR)	18 (8-58)	29 (13-65)	0.15
HAQ, median (IQR)	1.1 (0.8-1.5)	1.6 (1.4-2.1)	<0.01
DAS, mean (SD)	4.0 (0.8)	5.1 (0.7)	<0.01
SHS			
mean (SD)	6.7 (10.2)	5.7 (6.5)	
median (IQR)	3.5 (1.0-7.5)	4.0 (0.5-7.5)	0.91
Erosive disease, number (%)	43 (72)	28 (65)	0.48
Outcomes after 2 years of treatment			
Progression of joint damage, no (%)	6 (10)	15 (35)	<0.01
SHS progression			
mean (SD)	1.5 (3.5)	8.0 (18.9)	
median (IQR)	0.5 (0.0-1.5)	2.5 (0.0-9.5)	<0.01
HAQ, median (IQR)	0.1 (0.1-0.3)	1.3 (0.9-1.6)	<0.01

See table 1 for explanation of abbreviations.

Association of clinical improvement with functional ability and joint damage progression

Over 2 years follow-up, *remissions* had significantly better functional ability than *failures* and less progression of joint damage (Table 2). Compared with *remissions*, *failures* had 5 times higher risk of progressive disease as defined by progression score > SDC (OR 5.2 [95% CI 1.4 - 18.8]).

Over 2 years, functional ability was not significantly different in patients classified as *remissions* treated either with initial monotherapy or initial combination therapy (Table 3). Since *remissions* with initial combination therapy had significantly higher HAQ scores at baseline, the reduction in HAQ (ie, improvement in functional ability) was significantly greater (median [IQR] ΔHAQ -1.1 points [-1.6- -0.8]) for *remissions* treated with initial combination therapy than for *remissions* treated with initial monotherapy (median [IQR] ΔHAQ -0.6 [-1.0- -1.0]; $P < 0.01$). Over 2 years, functional ability was significantly higher for *failures* treated with initial monotherapy (median [IQR] HAQ 1.5 [1.0-1.8]) as compared with *failures* treated with initial combination therapy (1.0 [0.8-1.5]; $P =$

0.02). Mean DAS over 2 years was lower for *remissions* treated with initial combination therapy and for *failures* treated with initial combination therapy as compared with either subset treated with initial monotherapy (mean DAS [SD] *remissions* with monotherapy: 1.4 [0.3], *remissions* with combination therapy: 1.2 [0.3], $P = 0.02$; mean DAS [SD] *failures* with monotherapy: 3.8 [0.5], *failures* with combination therapy: 3.4 [0.5], $P = 0.01$).

Significantly less *remissions* treated with initial combination therapy showed progression of joint damage than *remissions* treated with initial monotherapy (3% vs 25% of *remissions* in both treatment groups, $P < 0.01$; Table 3). The percentage of patients with progressive disease was comparable between *failures* treated with initial monotherapy (38%) and *failures* treated with initial combination therapy (32%; $P = 0.67$). Mean and median progression scores were slightly higher for both *remissions* and *failures* with initial monotherapy as compared with *remissions* and *failures* with initial combination therapy (Table 3), but these differences were not statistically significant.

Additionally, the association of *remission* and *failure* with progression scores and functional ability for patients in group 3 (combination including prednisone) and patients in group 4 (combination including infliximab) were analyzed, but no differences were observed (Mean [SD]/ median [IQR] SHS *remissions* group 3: 1.2 [3.0]/ 0.5 [0.0-1.5]; *remissions* group 4: 0.7 [1.5]/ 0.5 [-0.3-1.5]; *failures* group 3: 3.6 [5.0]/ 0.8 [0.0-8.0]; *failures* group 4: 5.9 [6.2]/ 2.8 [1.1-13.4]. Percentage with progression: *remissions* group 3: 5%; *remissions* group 4: 0%; *failures* group 3: 25%; *failures* group 4: 40%. Median [IQR] HAQ: *remissions* group 3: 0.1 [0.1-0.3]; *remissions* group 4: 0.1 [0.1-0.3]; *failures* group 3: 1.0 [0.8-1.3]; f*ailures* group 4: 1.1 [0.8-1.6]. All *P*-values > 0.05).

Table 3. Number of *remissions* and *failures* and progression of joint damage and functional ability for *remissions* and *failures* on initial monotherapy and initial combination therapy

	Initial monotherapy	Initial combination therapy	*P* value
Remissions (n)	21	40	0.02
Progression of joint damage (%)	25	3	<0.01
SHS progression			
mean (SD)	2.5 (5.1)	0.9 (2.3)	
median (IQR)	0.3 (0.0-4.3)	0.5 (0.0-1.5)	0.81
HAQ, median (IQR)	0.2 (0.1-0.4)	0.1 (0.1-0.3)	0.59
Failures (n)	21	22	0.93
Progression of joint damage (%)	38	32	0.67
SHS progression			
mean (SD)	11.6 (26.3)	4.6 (5.6)	
median (IQR)	3.5 (0.3-11.0)	2.5 (0.0-10.0)	0.58
HAQ, median (IQR)	1.5 (1.0-1.8)	1.0 (0.8-1.5)	0.02

DISCUSSION

Initial treatment of RA with a combination of either methotrexate, sulphasalazine and a tapered high dose of prednisone or methotrexate with infliximab results in continuous remission in twice as many patients compared with initial monotherapy with methotrexate. Moreover, patients with continuous remission on initial combination therapy showed significantly less often progression of joint damage than patients with continuous remission on initial monotherapy, which suggests that combination therapy has an additional effect on joint damage prevention distinct from its effect on clinical disease activity.

In our study, the lack of clinical improvement despite treatment adjustments resulted in a 5 times higher risk for progression of joint damage over 2 years as compared with early, sustained remission. These data support earlier reports on a strong association between persistent disease activity and joint damage progression (1-3). Interestingly, in the BeSt study, patients who achieved continuous remission on initial combination therapy all tapered treatment to monotherapy after 9 months and still showed superior suppression of joint damage progression compared with patients who achieved continuous remission on initial monotherapy. Based on this finding, remission achieved through initial combination therapy with infliximab or prednisone may be different from remission achieved through initial MTX monotherapy. This may be, in part, due to the fact that patients treated with initial combination therapy achieved clinical remission earlier (38%, n = 15/40 at 3 months) than patients treated with initial monotherapy, and that clinical disease activity appears to be better suppressed.

Also , patients with continuous treatment failure benefited from initial combination therapy. Although the percentage of patients with progression was only slightly lower (32% vs 38%), a clear benefit in terms of functional ability was observed, again suggesting that initial combination therapy is superior to initial monotherapy.

We did not observe superior suppression of progression of joint damage in failures initially treated with MTX + infliximab, in contrast to the results of a subanalysis of the ATTRACT study (6). However, in that study, patients continued treatment with infliximab regardless of clinical response, whereas in the BeSt study, patients who did not achieve a good clinical response despite infliximab dose escalation, discontinued infliximab after an average of 8 months, and switched to sulphasalazine monotherapy (10,15). The observed suppression of progression of joint damage in patients without clinical response in the ATTRACT study, and the lack of progression of joint damage in remissions in the BeSt study who discontinued infliximab support the suggestion that suppression of progression of joint damage is a specific drug effect of infliximab. The same suppression effect is possibly true for prednisone. We did not find significant differences between patients treated with the combination including prednisone and patients treated with the combination including infliximab. However, this may be due to the small population of the current analysis.

In conclusion, we have observed that initial combination therapy with infliximab or prednisone is superior to initial methotrexate monotherapy resulting in a higher frequency of early and sustained remission, with less progression of joint damage, and better functional ability despite insufficient DAS reduction.

REFERENCES

1. Drossaers-Bakker KW, de Buck M, van Zeben D, Zwinderman AH, Breedveld FC, Hazes JM. Long-term course and outcome of functional capacity in rheumatoid arthritis: the effect of disease activity and radiologic damage over time. Arthritis Rheum 1999; 42(9):1854-1860.
2. van Leeuwen MA, van Rijswijk MH, Sluiter WJ, van Riel PL, Kuper IH, van de Putte LB et al. Individual relationship between progression of radiological damage and the acute phase response in early rheumatoid arthritis. Towards development of a decision support system. J Rheumatol 1997; 24(1):20-27.
3. Welsing PM, Landewe RB, van Riel PL, Boers M, van Gestel AM, van der Linden S. et al. The relationship between disease activity and radiologic progression in patients with rheumatoid arthritis: a longitudinal analysis. Arthritis Rheum 2004; 50(7):2082-2093.
4. Fransen J, Moens HB, Speyer I, van Riel PL. Effectiveness of systematic monitoring of rheumatoid arthritis disease activity in daily practice: a multicentre, cluster randomised controlled trial. Ann Rheum Dis 2005; 64(9):1294-1298.
5. Grigor C, Capell H, Stirling A, McMahon AD, Lock P, Vallance R et al. Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomised controlled trial. Lancet 2004; 364(9430):263-269.
6. Smolen JS, Han C, Bala M, Maini RN, Kalden JR, van der Heijde DM et al. Evidence of radiographic benefit of treatment with infliximab plus methotrexate in rheumatoid arthritis patients who had no clinical improvement: a detailed subanalysis of data from the anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study. Arthritis Rheum 2005; 52(4):1020-1030.
7. van den Berg WB, van Riel PL. Uncoupling of inflammation and destruction in rheumatoid arthritis: myth or reality? Arthritis Rheum 2005; 52(4):995-999.
8. Landewe RB, van der Heijde DM, Klareskog L, van Vollenhoven R, Fatenejad S. Disconnect between inflammation and joint destruction after treatment with etanercept plus methotrexate: results from the trial of etanercept and methotrexate with radiographic and patient outcomes. Arthritis Rheum 2006; 54(10):3119-3125.
9. Svensson B, Boonen A, Albertsson K, van der HD, Keller C, Hafstrom I. Low-dose prednisolone in addition to the initial disease-modifying antirheumatic drug in patients with early active rheumatoid arthritis reduces joint destruction and increases the remission rate: a two-year randomized trial. Arthritis Rheum 2005; 52(11):3360-3370.
10. Goekoop-Ruiterman YP, Vries-Bouwstra JK, Allaart CF, van Zeben D, Kerstens PJ, Hazes JM et al. Comparison of treatment strategies in early rheumatoid arthritis: a randomized trial. Ann Intern Med 2007; 146(6):406-415.
11. van Gestel AM, Prevoo ML, 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. Arthritis Rheum 1996; 39(1):34-40.
12. Fries JF, Spitz P, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. Arthritis Rheum 1980; 23(2):137-145.

13. van der Heijde DM. How to read radiographs according to the Sharp/van der Heijde method. Journal of Rheumatology 1999; 26(3):743-745.
14. Prevoo MLL, VanGestel AM, VantHof MA, VanRijswijk MH, VandePutte LBA, vanRiel PLCM. Remission in a prospective study of patients with rheumatoid arthritis. American rheumatism association preliminary remission criteria in relation to the disease activity score. British Journal of Rheumatology 1996; 35(11):1101-1105.
15. van de Bijl AE, van der Kooij SM, Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Breedveld FC, Allaart CF et al. Persistent good clinical response after tapering and discontinuation of initial infliximab therapy in patients with early rheumatoid arthritis: 3-year results from the BeSt-trial. Ann.Rheum.Dis. 65[Supplement II], 109. 1-7-2006.

Chapter 7

Ex vivo IL1Ra production upon LPS stimulation is associated with development of RA and with greater progression of joint damage

J. K. de Vries-Bouwstra
Y. P. M. Goekoop-Ruiterman
J. Wesoly
H. J. Hulsmans
A. J. M. de Craen
F. C. Breedveld
B. A. C. Dijkmans
C. F. Allaart
T. W. J. Huizinga

Ann Rheum Dis 2007 [Epub ahead of print; doi10.1136/ard.2006.062463]

ABSTRACT

Objectives 1) To assess innate ex vivo production of interleukin 1β (IL1β) and IL1 receptor antagonist (IL1Ra) in patients with recent-onset rheumatoid arthritis (RA) as compared to healthy controls, 2) to assess the association of ex vivo IL1β and IL1Ra production with progression of joint damage in RA, 3) to determine whether differences in ex vivo IL1β production are explained by distribution of the IL1β SNP C-511T.

Methods Levels of IL1β and IL1Ra (measured by ELISA after whole-blood stimulation with lipopolysaccharide [LPS]), and distribution of IL1β C-511T were compared in 76 disease-modifying antirheumatic drug (DMARD)-naive patients with recent-onset RA and 63 healthy controls. Odds ratios (OR) for RA based on ex vivo IL1β and IL1Ra production were calculated. Association of ex vivo IL1β and IL1Ra production with progression of joint damage (Sharp-van der Heijde score over 2 years) were determined by linear regression with correction for baseline characteristics.

Results Patients with recent-onset RA showed lower ex vivo IL1β and higher ex vivo IL1Ra production than healthy controls ($P < 0.001$), with OR for RA of 2.4 (95% confidence interval [CI] 1.2-4.9) for low IL1β-producers and 7.6 (95% CI 3.2-18.0) for high IL1Ra-producers. High ex vivo IL1Ra production was associated with joint damage progression ($P = 0.01$). The IL1β C-511T genotype distribution was not significantly different between patients and controls.

Conclusions Patients with recent-onset RA had decreased ex vivo IL1β production and increased ex vivo IL1Ra production compared to controls. Ex vivo IL1Ra production is an independent predictor of joint damage progression in recent-onset RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a complex disease in which both genetic and environmental factors play important etiologic roles (1). Cytokines play a pivotal role in the inflammatory process observed in RA and interindividual differences in the capacity to produce cytokines may be associated with susceptibility to and/or severity of RA (1,2). Studies of twins have shown that the heritability of ex vivo production of cytokines in whole blood induced by lipopolysaccharide (LPS) varies from 50% to 80% (3,4). The interindividual differences in the capacity to produce cytokines can be used as an endophenotype to enhance the elucidation of the genetic risk factors for RA (5-7).

There is ample evidence for the key role of the proinflammatory cytokine interleukin 1 beta (IL1β) in the pathogenesis of RA (8,9). Enhanced levels have been measured in synovial fluid and in plasma of RA patients and increased plasma levels of IL1β have shown a positive correlation with disease progression (10-12). Even low concentrations of IL1β can result in destruction of cartilage and bone (10). Interleukin-1 receptor antagonist (IL1Ra), a member of the IL1 family that binds to the IL1-receptors but does not induce an intracellular response, is the most important physiological regulator of IL1β activity (8,13). In vivo, the balance between IL1β and IL1Ra is important and a considerable excess of IL1Ra is needed to inhibit IL1β (8,9). Therefore, it is relevant to know whether the capacity to produce IL1Ra is different in patients as compared to controls.

The IL1 gene family, comprising IL1β, IL1α and IL1RN, has been located on the long arm of human chromosome 2 (band 2q13). Two polymorphisms in the IL1Ra gene (IL1RN) and three polymorphisms in the IL1β gene have been described, which are all in linkage disequilibrium (14). One polymorphism on the IL1β gene is located in the exon region (C+3954T) and two are located in the promoter region (C-511 T and T-31C) (14,15). The polymorphism C>T at position -511 in the IL1β gene has been suggested to be the tagging SNP of a haplotype associated with a two- to three-fold increase in LPS-induced ex vivo production of IL1β (15).

We designed a study to investigate whether differences in innate ex vivo production of IL1β and IL1Ra contribute to susceptibility and severity of RA. To this end it was studied whether patients with recent-onset RA have different IL1β and IL1Ra production upon in vitro stimulation with LPS as compared to healthy controls. It was investigated whether, in recent-onset RA, innate ex vivo production of IL1β and IL1Ra was associated with radiographic progression. In addition, it was investigated whether the haplotype tagged by C-511T was differently distributed in RA versus healthy controls and associated with different levels of ex vivo IL1β production.

METHODS

Patients and controls

Seventy-six patients with recent-onset RA (ACR 1987 criteria, symptom duration < 2 years) participating in the BeSt study and 63 healthy controls were included.

Details of the BeSt study have been published (16). Briefly, this randomized multicenter clinical trial compared four treatment strategies in 508 patients with recent-onset RA: 1. sequential monotherapy, 2. step-up combination therapy, 3. initial combination therapy with prednisone, and 4. initial combination therapy with infliximab. Patients had received no prior treatment with DMARDs, with the exception of antimalarials. In the present analysis, 76 patients, who were consecutively enrolled in the study at the Leiden University Medical Center (LUMC; n = 18 group 1; n = 20 group 2; n = 19 group 3; n = 19 group 4) were included.

The 63 healthy controls were recruited from 54 families who had participated in a previous study on multiple sclerosis (MS) and systemic lupus erythematosus (SLE). The control individuals were spouses and first-degree relatives of the patients with SLE or MS. For the current analysis all unrelated individuals (n = 63), preferably women of > 40 years of age, were recruited (17). The Medical Ethics Committee of the LUMC approved the protocols of both the studies. Patients and controls gave written informed consent for the current research.

Measurement of IL1β and IL1Ra

Peripheral blood samples were taken at the first visit at the outpatient clinic, prior to initiation of DMARD therapy. In the controls the peripheral blood samples were taken after a physical examination by a physician to confirm healthy state. Blood samples were collected in pyrogen-free heparinized tubes between 8 AM and 11 AM. The samples were cultured within 2 hours after collection with and without (negative control) LPS 10 ng/mL in 4 mL tubes for 24 hours, after which the supernatant was collected and stored at -70ºC. IL1β and IL1Ra were measured by ELISA (IL1β: Sanquin, IL1Ra: Biosource) at the same time in all samples in one batch. At the moment of sample collection patients had normal white cell blood counts.

Genotyping

The polymorphism C>T at position 511 (rs 16944) in the promoter region of the IL1β gene was typed in all patients and controls with DNA available (n = 72 patients; n = 61 controls). Primer sequences and PCR conditions were: forward primer 5' GGT AAC AGC ACC TGG TCT TGC-3'; reverse primer 5' GCA CAT ACT TTT CTT CAT TCA CTT C-3'; PCR-cycles: 95˚C for 5 minutes, followed by 35 cycles of 95˚C for 30 seconds, 55˚C for 1 minute and 72˚C for 30 seconds, followed by 10 minutes at 72˚C. PCR products were digested with AvaI at 37˚C for 90 minutes and digests were resolved on 2.5% agarose gels.

Samples were typed by visual examination of the present size fragments. Ten percent of all typings was repeated. The error rate was < 0.5%.

Radiographs

Radiographs of hands and feet at baseline and after 2 years of follow-up were available for 70 of the 76 patients. Radiographic progression was determined (Sharp-van der Heijde score [SHS]) by using the mean score of two physicians, who scored the radiographs paired, independently, in random order, blinded for clinical data (18). Median progression was 1.0 (interquartile range [IQR] 0.0-3.8), mean (SD) 4.7 (11.7), which was comparable to the progression observed in the other patients participating in the BeSt study ($P = 0.474$). Three groups of patients were defined (tertiles): 1) *nonprogressive RA*, progression score ≤ 0; 2) *mildly progressive RA*, progression score > 0 and ≤ 2; and 3) *severely progressive RA*, progression score > 2.

Statistical analysis

For comparing means the Students t-test and the Mann Whitney U/ Kruskal-Wallis tests were used where appropriate. Proportions were compared with the chi-square test. Genotype frequencies were tested for Hardy-Weinberg equilibrium.

To compare ex vivo IL1β and IL1Ra production levels between patients and controls with correction for age and gender logistic regression was performed. To this end patients were identified as high or low IL1β- and IL1Ra-producers with cut-off levels based on the median production levels in healthy controls.

In patients univariate and multivariate linear regression analysis was performed to describe the relation between radiographic progression and ex vivo IL1β and IL1Ra production with correction for baseline characteristics associated with more severe progression (age, gender, duration of symptoms, C-reactive protein [CRP], rheumatoid factor positivity, anti-CCP positivity [n = 68], number of painful joints [Ritchie Articular Index], swollen joint count, Health Assessment Questionnaire [HAQ]-score, visual analog scale [VAS] for morning stiffness, and total SHS [19,20]), and for treatment group (categorical). IL1β and IL1Ra were added to a multivariate regression model including significant variables resulting from a backwards selection procedure (stepwise removal of variables with $P > 0.10$).

RESULTS

Baseline characteristics

The baseline characteristics of the patients are shown in Table 1. At baseline, this subgroup of patients from the BeSt study did not differ from the other 432 patients with the exception of the number of painful joints (mean 16 for 76 patients vs 14 for the other

432 patients [P = 0.014]) and the period between diagnosis and inclusion (median 1.4 weeks for the 76 included patients vs 2.6 weeks for the other 432 patients [P = 0.001]; other data not shown).

Table 1. Baseline characteristics of RA patients (n = 76)

Age, years†	55.5 (15.3)
No. (%) female	51 (67)
Duration of symptoms, weeks ϕ	20.9 (13.5-33.9)
Time between diagnosis and inclusion, weeks ϕ	1.4 (0.5-3.9)
No. (%) IgM rheumatoid factor positive	48 (63.2)
ESR †	41 (29)
Ritchie Articular Index †	16 (8)
No. swollen joints †	14 (7)
HAQ †	1.3 (0.7)
Sharp-van der Heijde Score (SHS) ϕ *	3.0 (1.0-8.1)
No. (%) patients with erosive disease*	53 (72)
Progression of SHS over 2 years ϕ *	1.0 (0.0-3.8)

† Mean (SD); ϕ Median (IQR); * Radiographs at baseline and after 2 years of follow-up available for 70 patients. ESR = erythrocyte sedimentation rate.

Among the 63 controls 34 were female (54%; P = 0.114 compared to patients) and the mean (SD) age was 54.6 (11.9) years (P = 0.685 compared to patients).

Ex vivo IL1β and IL1Ra production

The ex vivo production of IL1β was significantly lower in patients than in healthy controls. The ex vivo IL1Ra production was significantly higher in patients than in healthy controls (Table 2).

Table 2. Ex vivo IL1β and IL1Ra production upon stimulation with LPS (pg/mL; median [interquartile range]) of recent-onset RA patients and healthy controls

Cytokine	RA Patients (n = 76)	Controls (n = 63)	P value
IL1β	1536 (606-2854) †	2773 (1487-4901)	< 0.001
IL1 Ra	29010 (20459–41195)	18234 (1438-22293)	< 0.001

† n = 75 patients

Subjects characterized by low ex vivo IL1β production or high ex vivo IL1Ra production had increased risk for diagnosis of RA (Table 3). A multivariate model including both IL1β production and IL1Ra production showed consistent associations of ex vivo IL1β production and IL1Ra production with the diagnosis of RA (data not shown).

Table 3. Odds ratio (95% confidence interval) for RA, given low ex vivo IL1β production and high ex vivo IL1Ra production, with correction for age and gender

Baseline variable	OR (95% confidence interval)
IL1β †	2.4 (1.2 - 4.9)
IL1 Ra ‡	7.6 (3.2 - 18.0)

† Level lower than median in controls
‡ Level higher than median in controls

To rule out possible effects of prescribed medication on the ex vivo production of IL1β and IL1Ra, it was tested whether in these DMARD-naive patients the ex vivo IL1β and IL1Ra production levels were associated with the use of nonsteroidal anti-inflammatory drugs (NSAIDs), paracetamol, opioids, or other concomitant medication used at time of blood sample collection. None of the medications were associated with ex vivo IL1β or IL1Ra production (data not shown).

Association of ex vivo IL1β and IL1Ra production with progression of joint damage

Nonprogressive RA patients (n = 26) had the lowest baseline ex vivo IL1β production (median [IQR] 1369 pg/mL [605-2804 pg/mL]); followed by patients with mildly progressive RA (n = 22; 1611 pg/mL [636–2176 pg/mL]) and then by patients with severely progressive RA (n = 22; 1881 pg/mL [422-4000 pg/mL]; $P = 0.811$ for comparison among the three groups).

The patients with severely progressive RA had the highest baseline ex vivo IL1Ra production, of 31516 pg/mL (27677-46098 pg/mL), which was significantly higher than in patients with nonprogressive (22158 pg/mL [14863-35289 pg/mL]) and mildly progressive RA (30208 pg/mL [23042-43365 pg/mL]; $P = 0.03$ for comparison among the three groups).

In the univariate linear regression analysis, treatment group and total SHS at baseline were significantly associated with radiographic progression. Following these ex vivo IL1Ra production had the highest explained variance ($R^2 = 4.4\%$). The backward selection procedure identified the following clinical variables as significantly contributing to radiographic progression: treatment group, CRP, swollen joint count, HAQ, VAS for morning stiffness, and total SHS. Adding ex vivo IL1β and/or IL1Ra production to the multivariate model including these variables showed that IL1Ra was significantly associated with radiographic progression after correction for these variables (Table 4).

Table 4. Association of ex vivo IL1β and IL1Ra production and baseline disease characteristics with progression of joint damage; univariate and multivariate linear regression; regression coefficients, R2† and *P* values

Baseline variables	Univariate analysis			Multivariate analysis *	
	Standardized coefficient	$R^{2\,\dagger}$	*P* value	Standardized coefficient	*P* value
Age	-0.163	0.026	0.179	-	-
Gender	-0.187	0.035	0.121	-	-
IL1β	-0.046	0.002	0.710	-0.05	0.715
IL1Ra	0.209	0.044	0.082	-0.274	0.036
Treatment group ‡	-0.382	0.146	0.001	-0.399	0.002
Duration of complaints	0.055	0.003	0.651	-	-
CRP	0.059	0.003	0.648	0.151	0.276
Anti-CCP	0.154	0.024	0.229	-	-
Rheumatoid factor	0.125	0.016	0.304	-	-
Ritchie Articular Index	-0.078	0.006	0.520	-	-
Total swollen joint count	-0.142	0.02	0.242	-0.042	0.747
HAQ	0.165	0.027	0.174	-0.087	0.517
VAS morning stiffness	0.032	0.001	0.794	-0.101	0.459
SHS	0.327	0.107	0.007	-0.151	0.118

† R^2 = Explained variance; * Multivariate linear regression analysis including the variables selected by backward selection procedure and IL1β and IL1Ra.
‡ Treatment groups are: sequential monotherapy, step-up combination therapy, initial combination therapy with prednisone, and initial combination therapy with infliximab.

IL1β C-511T in patients and controls

Genotype frequencies showed a distribution in accordance with Hardy-Weinberg equilibrium for both patients and controls. Although the frequency of the T-allele was slightly higher in the patients, no significant differences were observed in the genotype or allele distribution between patients and controls (Table 5).

Table 5. Genotype and allele frequencies in patients and controls

	IL1β C-511T				
	Genotype			Allele frequency	
	CC	CT	TT	C	T
Patients (n = 72)	31 (43.1%)	30 (41.7%)	11 (15.3%)	0.64	0.36
Controls (n = 61)	30 (49.2%)	27 (44.3%)	4 (6.6%)	0.71	0.29

Comparisons of genotype distribution between patients and controls: for CC, CT, TT, *P* = 0.28; for CC and CT versus TT, *P* = 0.11; for CC versus CT and TT, *P* = 0.48 ; and for allele frequency, *P* = 0.29.

For controls with the T-allele, a trend was observed for higher ex vivo production of IL1β (median IL1β production 2666 pg/mL for CC, 2801 pg/mL for CT, and 3670 pg/mL for TT; $P = 0.078$); in patients this correlation was not observed. There was no difference in the distribution of the IL1β genotype among the subgroups of patients with nonprogressive, mildly progressive and severely progressive RA.

DISCUSSION

The ex vivo LPS-induced production of IL1β and IL1Ra was observed to be significantly different between recent-onset RA patients and healthy controls. Intriguingly, the RA patients produced less IL1β and more IL1Ra than controls in this assay. For the first time, this study showed that higher ex vivo IL1Ra production at baseline was associated with higher radiographic progression.

The current assay demonstrates a specific ex vivo cytokine production profile that is associated with diagnosis and severity of RA indicating a specific endophenotype of recent-onset RA. Determination of endophenotypes is a useful and novel method to target further research regarding the genetic background of multifactorial diseases (6). In line with the current observations, a recently published paper on gene expression profiles in RA identified IL1Ra as one of the top discriminators between peripheral blood mononuclear cells of healthy controls and RA patients (21). Hence, the importance of IL1Ra in RA has been underscored by two completely different approaches.

The observation that RA patients have increased ex vivo IL1Ra production and decreased ex vivo IL1β production seems paradoxical. The following considerations have to be made.

First, IL1β and IL1Ra production were determined in vitro using a powerful stimulator to maximize cytokine production. It is unclear how the measured maximum production capacity in vitro is related to actual (circulating) levels in RA patients. Several reports in the field of cytokines have shown that observations made in vitro may not mimic the in vivo situation since multiple micro-environmental factors are important for cytokine production (22). However, since clear differences are observed between healthy controls and RA patients, these results point at crucial alterations in the regulation of IL1β and IL1Ra production in RA which can be caused by a polymorphism in a transcription factor or in of one of the LPS receptors.

Second, the biological relevance of high IL1Ra levels in vivo is unclear, because a 10- to 500-fold excess of IL1Ra over IL1β is needed to decrease the stimulation of target cells (23,24). Thus, the elevated levels of IL1Ra may not be sufficient to affect the effects of IL1β (25). It is likely that IL1Ra production increases in response to IL1β production to counterbalance the proinflammatory effects of IL1β (25). If so, IL1Ra seems to be a more reliable indicator of disease activity than IL1β itself, possibly because of the strong signal peptide and the caspase-1 independent production (26).

The reliability of the assay used to determine interindividual differences in cytokine production has been extensively demonstrated (7). Regression analysis was performed to correct for age and gender (27), and a possible effect of prescribed medication in patients

on cytokine production was ruled out. Therefore, the described method is likely to be reliable to describe cytokine production profiles in recent-onset RA.

The heritability of ex vivo cytokine production was underscored in a twin study; estimates of heritability of IL1β and IL1Ra production were 86% and 53%, respectively (3). Nevertheless, the difference in IL1β production between RA patients and healthy controls could not be explained by the distribution of C-511T. This is in contrast to a previous study which found a specific haplotype tagged by C-511T to be associated with higher IL1β production in patients (n = 25) and in controls (n = 31) (15). Compared to the mentioned study, the results in the healthy control group (n = 61) seem to confirm these data as there was a trend for higher IL1β production with carriership of the T-allele. Since no association was observed in the RA patients (n = 72), we do not know whether the difference in innate IL1β production is caused by a mutation in the IL1 genes. In general, studies concerning the association of IL1 gene polymorphisms with IL1β production have shown different and conflicting results (14,28-31). Probably, other related polymorphisms and/or epigenetic factors, like methylation of genes, contribute to different levels of innate IL1β production.

A strength of the current study is that blood samples were obtained very early in the disease course of 76 patients with severe RA (16). RemarkabIy, ex vivo IL1Ra production was a better predictor of radiographic progression than several well-known predictors of destructive RA (19,20). The association of IL1Ra production with joint damage despite aggressive treatment and low overall progression scores in the cohort under study points at a key role for IL1β and IL1Ra in the pathogenesis of RA.

In conclusion, the results of this study show that ex vivo LPS-induced IL1β and IL1Ra production levels indicate a RA-specific endophenotype with high ex vivo production of IL1Ra being independently and strongly associated with radiographic progression. Further studies addressing the pathogenetic background of high ex vivo IL1Ra production in recent-onset RA are needed and useful to unravel the genetic background of RA.

REFERENCES

1. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature. 2003;423:356-361.
2. Klimiuk PA, Goronzy JJ, Bjor nJ, Beckenbaugh RD, Weyand CM. Tissue cytokine patterns distinguish variants of rheumatoid synovitis. Am J Pathol. 1997;151:1311-1319.
3. De Craen AJ, Posthuma D, Remarque EJ, Van Den Biggelaar AH, Westendorp RG, Boomsma DI. Heritability estimates of innate immunity: an extended twin study. Genes Immun. 2005;6:167-170.
4. Westendorp RG, Langermans JA, Huizinga TW, Elouali AH, Verweij CL, Boomsma DI et al. Genetic influence on cytokine production and fatal meningococcal disease. Lancet. 1997;349:170-173.
5. de Jong BA, Huizinga TW, Bollen EL, Uitdehaag BM, Bosma GP, van Buchem MA et al. Production of IL-1beta and IL-1Ra as risk factors for susceptibility and progression of relapse-onset multiple sclerosis. J Neuroimmunol. 2002;126:172-179.
6. Savitz JB, Cupido CL, Ramesar RS. Trends in suicidology: personality as an endophenotype for molecular genetic investigations. PLoS Med. 2006;3:e107.
7. van der Linden MW, Huizinga TW, Stoeken DJ, Sturk A, Westendorp RG. Determination of tumour necrosis factor-alpha and interleukin-10 production in a whole blood stimulation system: assessment of laboratory error and individual variation. J Immunol Methods. 1998;218:63-71.
8. Dayer JM. Evidence for the biological modulation of IL-1 activity: the role of IL-1Ra. Clin Exp Rheumatol. 2002;20:S14-S20.
9. Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. Annu Rev Immunol. 1996;14:397-440.
10. Dayer JM. The pivotal role of interleukin-1 in the clinical manifestations of rheumatoid arthritis. Rheumatology (Oxford). 2003;42 Suppl 2:ii3-10.
11. Eastgate JA, Symons JA, Wood NC, Grinlinton FM, di Giovine FS, Duff GW. Correlation of plasma interleukin 1 levels with disease activity in rheumatoid arthritis. Lancet. 1988;2:706-709.
12. North J, Situnayake RD, Tikly M, Cremona A, Nicoll J, Kumararatne DS et al. Interleukin 1 beta, hand and foot bone mineral content and the development of joint erosions in rheumatoid arthritis. Ann Rheum Dis. 1994;53:543-546.
13. Dinarello CA. The many worlds of reducing interleukin-1. Arthritis Rheum. 2005;52:1960-1967.
14. Buchs N, di Giovine FS, Silvestri T, Vannier E, Duff GW, Miossec P. IL-1B and IL-1Ra gene polymorphisms and disease severity in rheumatoid arthritis: interaction with their plasma levels. Genes Immun. 2001;2:222-228.
15. Hall SK, Perregaux DG, Gabel CA, Woodworth T, Durham LK, Huizinga TW et al. Correlation of polymorphic variation in the promoter region of the interleukin-1 beta gene with secretion of interleukin-1 beta protein. Arthritis Rheum. 2004;50:1976-1983.
16. Goekoop-Ruiterman YPM, De Vries-Bouwstra JK, Allaart CF, van Zeben D, Kerstens PJSM, Hazes JMW et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt Study). Arthritis Rheum. 2005;52:3381-3390.
17. van der Linden MW, Westendorp RG, Sturk A, Bergman W, Huizinga TW. High interleukin-10 production in first-degree relatives of patients with generalized but not cutaneous lupus erythematosus. J Investig Med. 2000;48:327-334.

18. van der Heijde D. How to read radiographs according to the Sharp/van der Heijde method. J Rheumatol. 2000;27:261-263.
19. Jansen LM, van der Horst-Bruinsma IE, van Schaardenburg D, Bezemer PD, Dijkmans BA. Predictors of radiographic joint damage in patients with early rheumatoid arthritis. Ann Rheum Dis. 2001;60:924-927.
20. Scott DL. Prognostic factors in early rheumatoid arthritis. Rheumatology (Oxford). 2000;39 Suppl 1:24-29.
21. Batliwalla FM, Baechler EC, Xiao X, Li W, Balasubramanian S, Khalili H et al. Peripheral blood gene expression profiling in rheumatoid arthritis. Genes Immun. 2005;6:388-397.
22. Crispin JC, Alcocer-Varela J. Interleukin-2 and systemic lupus erythematosus--fifteen years later. Lupus 1998; 7:214-222.
23. Malyak M, Swaney RE, Arend WP. Levels of synovial fluid interleukin-1 receptor antagonist in rheumatoid arthritis and other arthropathies. Potential contribution from synovial fluid neutrophils. Arthritis Rheum. 1993;36:781-789.
24. Shingu M, Fujikawa Y, Wada T, Nonaka S, Nobunaga M. Increased IL-1 receptor antagonist (IL-1ra) production and decreased IL-1 beta/IL-1ra ratio in mononuclear cells from rheumatoid arthritis patients. Br J Rheumatol. 1995;34:24-30.
25. Arend WP, Malyak M, Guthridge CJ, Gabay C. Interleukin-1 receptor antagonist: role in biology. Annu Rev Immunol. 1998;16:27-55.
26. Fantuzzi G. Lessons from interleukin-deficient mice: the interleukin-1 system. Acta Physiol Scand. 2001;173:5-9.
27. Pietschmann P, Gollob E, Brosch S, Hahn P, Kudlacek S, Willheim M et al. The effect of age and gender on cytokine production by human peripheral blood mononuclear cells and markers of bone metabolism. Exp Gerontol. 2003;38:1119-1127.
28. El Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature. 2000;404:398-402.
29. Hwang IR, Kodama T, Kikuchi S, Sakai K, Peterson LE, Graham DY et al. Effect of interleukin 1 polymorphisms on gastric mucosal interleukin 1beta production in Helicobacter pylori infection. Gastroenterology. 2002;123:1793-1803.
30. Read RC, Camp NJ, di Giovine FS, Borrow R, Kaczmarski EB, Chaudhary AG et al. An interleukin-1 genotype is associated with fatal outcome of meningococcal disease. J Infect Dis. 2000; 182:1557-1560.
31. Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. Scand J Immunol. 1998;47:195-198.

Chapter 8

Efficacy and toxicity of methotrexate in early rheumatoid arthritis are associated with Single-Nucleotide Polymorphisms in genes coding for folate pathway enzymes

J. A. M. Wessels*
J. K. de Vries-Bouwstra*
B. T. Heijmans
P. E. Slagboom
Y. P. M. Goekoop-Ruiterman
C. F. Allaart
P. J. S. M. Kerstens
D. van Zeben
F. C. Breedveld
B. A. C. Dijkmans
T. W. J. Huizinga
H.-J. Guchelaar

Arthritis Rheum 2006; 54(4): 1087-95

*Both authors contributed equally to this study

ABSTRACT

Objective To determine associations of methotrexate (MTX) efficacy and toxicity with single-nucleotide polymorphisms (SNPs) in genes coding for folate pathway enzymes in patients with early rheumatoid arthritis (RA).

Methods Patients (n = 205) with active RA received MTX at an initial dosage of 7.5 mg/wk, which was increased to 15 mg/wk and combined with folic acid (1 mg/day) after 4 weeks. If the Disease Activity Score in 44 joints (DAS) was > 2.4 at 3 months, MTX was increased to 25 mg/wk. MTX efficacy was evaluated at 3 and 6 months and compared for genotypes in 3 analyses: patients with and without good response (DAS ≤ 2.4), patients with and without good improvement (ΔDAS > 1.2), and patients with and without moderate improvement (ΔDAS > 0.6). The association between MTX-related adverse drug events (ADEs) and genotype was evaluated by comparing genotypes between patients with and without ADEs, specifically pneumonitis, gastrointestinal ADEs, skin and mucosal ADEs, and elevated liver enzyme levels. The following SNPs were analyzed: methylenetetrahydrofolate reductase (MTHFR) 677C>T, MTHFR 1298A>C, dihydrofolate reductase (DHFR) -473G>A, DHFR 35289G>A, and reduced folate carrier (RFC) 80G>A. In case of significant differences, odds ratios (ORs) were calculated.

Results At 6 months, MTHFR 1298AA was associated with good improvement relative to 1298C (OR 2.3, 95% confidence interval [95% CI] 1.18–4.41), which increased with increased copies of the MTHFR 677CC haplotype. In contrast, MTHFR 1298C allele carriers developed more ADEs (OR 2.5, 95% CI 1.32–4.72).

Conclusion Patients with MTHFR 1298AA and MTHFR 677CC showed greater clinical improvement with MTX, whereas only the MTHFR 1298C allele was associated with toxicity. In the future, MTHFR genotypes may help determine which patients will benefit most from MTX treatment.

INTRODUCTION

Methotrexate (MTX) is the most widely used disease-modifying antirheumatic drug (DMARD) for the treatment of rheumatoid arthritis (RA), and has proven to reduce disease activity and delay or stabilize the development of bone erosions (1,2). However, only ~50% of the patients experience good clinical response, and 30% discontinue therapy due to side effects (3,4). Demographic factors such as age and sex and factors such as disease duration, prior DMARD use, and folic acid supplementation have been studied to predict treatment outcome, but results regarding both efficacy and toxicity are conflicting (3,5–8).

However, it is highly appropriate to start directly with adequate therapy in RA to reduce joint damage and functional decline. The importance of early and sustained suppression of disease activity in RA has been shown in several randomized clinical trials (9–13). In this context, pharmacogenetics studies may offer a novel strategy to identify predictors of response and toxicity of MTX treatment (14–17).

Although the precise mechanism of action of MTX is unknown, it is believed to be related to the inhibition of folate pathway enzymes, including dihydrofolate reductase (DHFR), methylenetetrahydrofolate reductase (MTHFR), reduced folate carrier (RFC), thymidylate synthase (TS), and 5-aminoimidazole-4- carboxamide ribonucleotide (AICAR) transformylase (18,19). Several studies have shown associations of polymorphisms in the gene coding for MTHFR with MTX toxicity. MTHFR 677C>T was found to be associated with elevated levels of liver enzymes, hyperhomocysteinemia, and overall MTX toxicity (20–22). The MTHFR 1298A>C single-nucleotide polymorphism (SNP) has been found to be associated with the combined MTX-related gastrointestinal, hematologic, and mucosal adverse drug events (ADEs) (23). In combined analysis with MTHFR 677C>T, the MTHFR 1298A>C SNP was found to be associated with requirements for lower dosages of MTX in order to reduce disease activity (22). For MTX efficacy, an association of combined SNPs G>A in the RFC at position 80, C>G in AICAR transformylase position 347, and a SNP in the enhancer region of thymidylate synthase was suggested (24,25). In contrast, investigators in other studies were unable to confirm the associations of MTHFR 677C>T and 1298A>C with efficacy and toxicity of MTX in RA (20,26).

The aim of the current study was to determine associations between efficacy and toxicity of MTX and SNPs in genes coding for the folate pathway enzymes MTHFR, DHFR, and RFC in patients with early RA who were treated according to a strict protocol to achieve maximum suppression of disease activity.

PATIENTS AND METHODS

RA patient population and treatment

The group of 247 patients enrolled in this study was a subcohort of 508 patients who participated in the BeSt (Behandelstrategieën voor Reumatoïde Artritis) study (27), which is a 2-year, multicenter, single-blind, randomized clinical trial designed to compare the clinical efficacy of four different treatment strategies in early RA. The patients were diagnosed as having RA as defined by the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 revised criteria (28). Patients had to have a disease duration of < 2 years, had to be ≥ 18 years of age, and had to have active disease, with ≥ 6 swollen joints (66 joint count) and ≥ 6 tender joints (68 joint count) and either an erythrocyte sedimentation rate (ESR) ≥ 28 mm/hour or a visual analog scale for global health or a global health score of ≥ 20 mm on a 0-100-mm visual analog scale (0 = best and 100 = worst). Exclusion criteria included previous treatment with DMARDs other than antimalarial agents, concomitant treatment with an experimental drug, a malignancy within the last 5 years, bone marrow hypoplasia, a serum aspartate aminotransferase/alanine aminotransferase (AST/ ALT) level > 3 times the upper limit of normal, a serum creatinine level > 150 μmoles/liter or an estimated creatinine clearance of < 75 ml/ minute, diabetes mellitus, alcohol or drug abuse, pregnancy or the wish to become pregnant during the study period, or inadequate contraception.

All patients were assigned to one of four treatment groups: group 1 (n = 126), sequential monotherapy, starting with MTX; group 2 (n = 121), step-up combination therapy, starting with MTX; group 3 (n = 133), initial combination therapy with MTX, sulphasalazine (SSZ), and high-dose prednisone; or group 4 (n = 128), initial biologic therapy with infliximab and MTX. The local ethics committee approved this study. Informed consent was obtained from all patients.

In this study, only the patients assigned to groups 1 and 2, characterized by first-line single use of MTX, were analyzed (n = 247). These patients started with MTX at a dosage of 7.5 mg/wk, then it was increased to 15 mg/wk after 4 weeks, and was combined with 1 mg/day folic acid. In both groups, treatment was aimed at good clinical response, as defined by a Disease Activity Score in 44 joints (DAS) of ≤ 2.4 (29,30). A research nurse who remained blinded with regard to the assigned treatment group assessed the DAS every 3 months. In case of insufficient clinical response at 3 months of follow-up (DAS > 2.4), the MTX dosage was increased by 5 mg every 2 weeks, to a maximum of 25 mg/week. If the clinical response remained insufficient at 6 months of follow-up with MTX 25 mg/week, patients were treated according to the next step of the protocol: patients in group 1 were switched to 1,000 mg SSZ twice a day, and for patients in group 2, 1,000 mg SSZ twice a day was added to MTX 25 mg/wk. In the current retrospective study, clinical data from the first 6 months of follow-up were used. In case of ADEs, MTX was continued at the

lowest tolerated dosage, or if MTX was not tolerated at all, the patient was treated according to the next treatment step. Concomitant therapies with nonsteroidal antiinflammatory drugs as well as intraarticular injections with corticosteroids were allowed for all treatment groups.

DNA samples could be obtained from 205 of the 247 patients randomized to initial MTX monotherapy. No statistically significant differences were found in baseline characteristics between the 205 genotyped patients and the 42 patients who were not genotyped ($P > 0.05$). Of the 205 patients with DNA available, 4 patients did not have efficacy analyses performed at 3 months: 1 patient had moved, 1 patient refused to take MTX after a short period of treatment with no ADEs, and 2 patients discontinued treatment after actually experiencing ADEs. Therefore, complete clinical data at 3 months of follow-up were obtained for 201 of 205 patients. At 6 months, data on 15 additional patients were missing: 1 patient had moved, in 5 patients the DAS was not calculated, 1 patient began taking SSZ before evaluation, and 8 patients had discontinued MTX permanently after experiencing ADEs. Therefore, the overall number of patients available for efficacy evaluation at 6 months was 186.

For toxicity analysis; all 205 genotyped patients were included for analysis at 3 months. Data on 5 patients were defined as missing at 6 months: 4 patients did not present at follow-up, and 1 patient had moved. Therefore, the overall number of patients for whom toxicity data were available at 6 months was 200. In patients who switched to therapy other than MTX before 6 months of follow-up, ADEs after this change of therapy were verified, and the patients were included in the toxicity analysis.

Efficacy evaluation

To monitor response to treatment and provide guidelines for dosage adjustments, the European League Against Rheumatism response criteria based on the DAS were used (29,30). Responders to MTX therapy were defined as patients with a DAS ≤ 2.4, and nonresponders were defined as patients with a DAS > 2.4. The efficacy of MTX was evaluated at 3 and 6 months by comparing the genotype distribution with clinical response in 3 analyses: patients with and without good clinical response, defined as DAS ≤ 2.4; patients with and without good improvement, defined as ΔDAS > 1.2; and patients with and without moderate improvement, defined as ΔDAS > 0.6.

Toxicity evaluation

For evaluation of toxicity, all reported ADEs during 3 and 6 months of MTX treatment were used. ADEs were reported by the patients themselves, or were reported as a result of nonspecific questioning on patients' well-being by the investigator, physical examination, or laboratory measurements during follow-up. Each adverse event was described by its duration, frequency, severity, an assessment of its cause,

its relationship to the study medication, whether it influenced the course of treatment, and whether it required specific therapy. In general, the dosage of MTX was lowered temporarily in case of mild ADEs (mild gastrointestinal symptoms, mild mucositis, persistent elevation of AST/ALT to < 3 times the upper limit of normal, persistent leukopenia [3–3.5 x 10^9 cells/liter], or thrombocytopenia [100–150 x 10^9 cells/liter]), and reintroduced at the maximum tolerated dosage after the ADE had resolved. In case of a severe ADE (severe gastrointestinal symptoms, severe mucositis, pneumonitis, elevation of AST/ALT to ≥ 3 times the upper limit of normal, leucopenia [< 3.0.10^9 cells/liter], or thrombocytopenia [< 50–100. 10^9 cells/liter]), MTX was discontinued, and was later reintroduced depending on the clinical situation. If MTX was not tolerated at all or was contraindicated, it was withdrawn and patients were treated according to the next step in the protocol.

Of all reported ADEs, the following noninfectious ADEs were evaluated explicitly: gastrointestinal ADEs, defined as patients' general well-being, nausea, vomiting, diarrhea, and constipation; liver ADEs, defined as all cases of elevated liver enzyme levels resulting in MTX dosage adjustment or discontinuation; pneumonitis; and skin and mucosal disorders. Furthermore, patients were evaluated at 3 and 6 months for leucopenia < 4x10^9 cells/liter, for ALT > 3 times the upper limit of normal (> 135 units/liter), and for alkaline phosphatase > 3 times the upper limit of normal (> 360 units/liter).

Measurement of SNPs in MTHFR, DHFR, and RFC genes

Five SNPs in 3 genes coding for MTHFR, DHFR, and RFC (rs1801133, rs1801131, rs1051266, rs1650697, and rs1232027) were selected from the National Center for Biotechnology Information SNP database (www.ncbi.nlm.nih.gov) using the following criteria: genotype frequency of ~10% or more, validated SNP, SNP-caused nonsynonymous amino acid change, and indications for clinical relevance determined from previous publications. The following SNPs were analyzed: MTHFR 677C>T, MTHFR 1298A>C, DHFR -473G>A (DNA alignment), DHFR 35289A>G (DNA alignment), and RFC 80G>A.

DNA was isolated from peripheral white blood cells by a standard manual salting-out method. MassARRAY assay designer software (Sequenom, San Diego, CA) was used to design multiplex genotyping assays (31). Genotyping was performed using the MassARRAY platform according to protocols provided by the manufacturer. Missing genotypes were analyzed using the PSQ 95MA pyrosequencing system (Biotage, Uppsala, Sweden) (32). The mean overall success rate was 97% and, more specifically, 100% for MTHFR 677C>T, 99% for MTHFR 1298A>C, 98% for DHFR 473G>A, 89% for DHFR 35289G>A, and 99% for RFC 80G>A.

Statistical analysis

All quantitative data are expressed as the mean ± SD unless specified otherwise. All qualitative data are expressed as frequencies and percentages. Differences in baseline characteristics were compared using Student's *t*-test or the chi-square test. Differences in genotype distribution for efficacy and toxicity were tested using 3 x 2 crosstabs for each genotype, and using 2 x 2 crosstabs for each possible combination of homozygote and heterozygote genotypes, with the 2-sided chi-square test.

In case of differences in genotype distribution between responders and nonresponders or between patients with and without ADEs, binary logistic analysis was performed to calculate the odds ratio (OR) for efficacy or toxicity of MTX given a certain genotype. Age and sex were identified as possible confounders and were used as covariates in all regression analyses.

Additionally, for efficacy analyses, the following possible confounders were identified: DAS at baseline, duration of joint symptoms before enrollment, duration of RA diagnosis before enrollment, rheumatoid factor (RF) positivity, Sharp-van der Heijde score (33) at baseline, Ritchie Articular Index (RAI) (34) at baseline, and C-reactive protein level. In multiple stepwise backward analyses, only DAS at baseline and RF positivity showed significant association with efficacy. These 2 covariates were taken into account in the efficacy logistic regression. The toxicity regression analysis was tested for the following confounders: weight, creatinine clearance, MTX dosage group (15 or 25 mg/week), and use of alcohol. None of the possible confounders for MTX-related adverse reactions showed a significant correlation with toxicity. Analyses of laboratory measurements were performed for study completers only.

A statistically significant difference was found between baseline characteristics of patients who were responders (defined as DAS ≤ 2.4) and those who were nonresponders (defined as DAS > 2.4) 6 months after starting MTX treatment. The nonresponder group had a higher percentage of women (77.2%), a higher RAI (median 16 versus 11), ESR (median 40 versus 34), and swollen joint count (median 14 versus 11), and a longer period of time between diagnosis of RA and inclusion in the BeSt study (median 2.57 weeks versus 1.71 weeks) when compared with responders. All other variables evaluated were shown to be not significantly different ($P > 0.05$). The only borderline difference for responders and nonresponders when defined as $\Delta DAS > 1.2$ or $\Delta DAS \leq 1.2$ at 6 months after starting MTX was the baseline Sharp-van der Heijde score (median 3 in responders versus 5 in nonresponders; $P = 0.047$). All statistical analyses were performed using SPSS software (version 11.5 for Windows; SPSS, Chicago, IL). Because 3 hypotheses were tested, a Bonferroni correction was performed for multiple comparisons. Both adjusted and unadjusted *P* values are presented. *P* values less than 0.05 were considered significant.

Table 1. Baseline characteristics of 205 genotyped patients *

Characteristic	Baseline value
Sex, % female / %male	68.8 / 31.2
Age, mean (SD) years	54.6 (13.3)
Disease duration, median (range) weeks	2.0 (0-104.7)
Duration of joint complaints,median (range) weeks	25.0 (1.1- 584.3)
DAS, mean (SD)	4.5 (0.8)
ESR, median (range) mm/hour	38 (2-143)
RF positivity,%	67.3
CRP, median (range) mg/liter	23 (0-238)
RAI, median (range)	13 (2-47)
No. of swollen joints, median (range)	13 (3-36)
Sharp-van der Heijde score, median (range)	4 (4-49.5)

* DAS = Disease Activity Score in 44 joints, ESR = Erythrocyte Sedimentation Rate
RF = rheumatoid factor, CRP = C-reactive protein, RAI = Ritchie Articular Index.

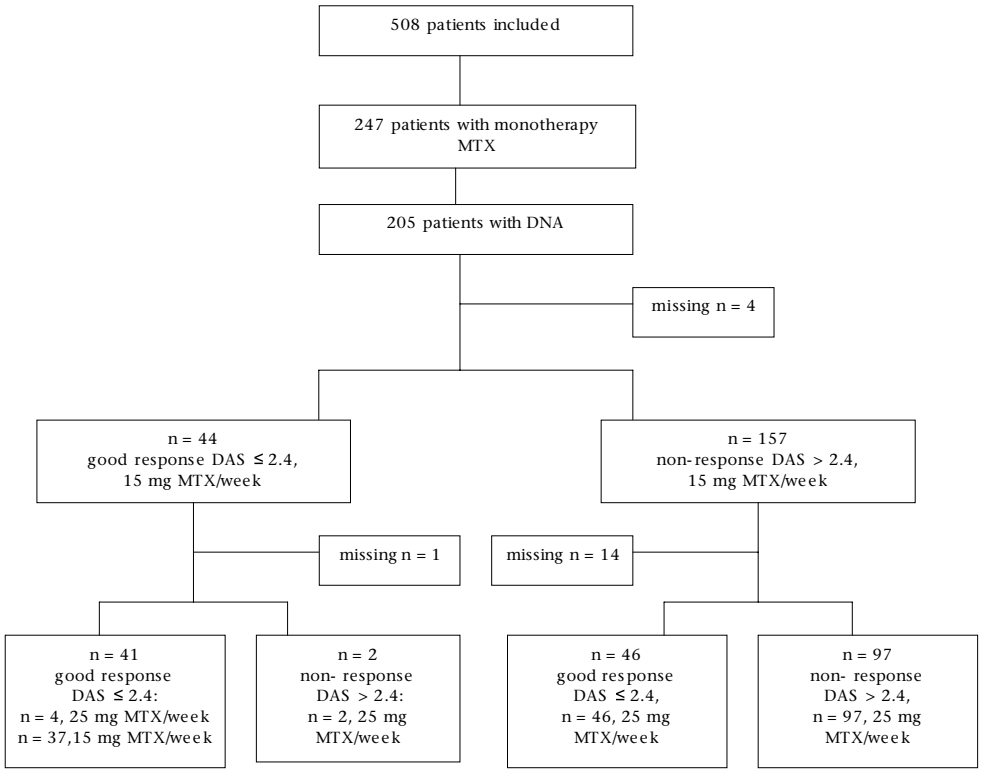


Figure 1. Flow chart of patient response after 3 and 6 months of methotrexate (MTX) treatment Responders achieved a Disease Activity Score in 44 joints (DAS) of ≤ 2.4 and nonresponders had a DAS > 2.4.

RESULTS

Description of the cohort

The baseline characteristics of our study population (n = 205) were comparable with those of other early RA cohorts (8,11). Clinical and demographic data are presented in Table 1. The study population was 93.2% white (n = 191), 2.4% Asian (n = 5), 1.0% black (n = 2), and 3.4% other (3 Hindustani, 3 Surinamese, and 1 Israeli). At 3 months, 22% of the patients (n = 44) were good responders. At 6 months, the percentage of patients achieving DAS ≤ 2.4 increased to 47% (n = 87), 43% of whom were taking 15 mg MTX/wk and 57% of whom were taking 25 mg MTX/wk. The total percentage of nonresponders at the 6-month follow-up was 53% (n = 99) (Figure 1).

Regarding toxicity, 21.5% (n = 44) and 34% (n = 68) of the study population experienced at least 1 ADE within 3 and 6 months of treatment, respectively, with most of the ADEs being mild or moderate. Eleven patients discontinued MTX permanently, for the following reasons: elevated liver enzyme levels (3 patients), gastrointestinal symptoms (3 patients), skin or mucosal disorders (3 patients), or other reasons (2 patients). Fourteen patients discontinued MTX temporarily due to elevated liver enzymes (9 patients), gastrointestinal symptoms (3 patients), skin or mucosal disorders (1 patient), or other reasons (1 patient). Of all patients (including those who ceased the MTX treatment temporarily or permanently), 23 patients did not tolerate the prescribed dosage of MTX (due to elevated liver enzyme levels [10 patients], gastrointestinal symptoms [6 patients], skin or mucosal disorders [2 patients], or other reasons [5 patients]). Three patients switched to parenteral MTX because of gastrointestinal side effects with oral MTX. In Table 2, frequencies and types of ADEs are indicated for all genotyped patients.

Genotype distributions were as follows: for MTHFR 677C>T 45% CC, 46% CT, 9% TT; for MTHFR 1298A>C 41% AA, 47% AC, 11% CC; for DHFR 35289G>A 48% GG, 40% GA, 12% AA; for DHFR -473G>A 60% GG, 37% GA, 3% AA; and for RFC80G>A 33% GG, 48% GA, 19% AA. There were no differences in allele distribution when compared with those in previous publications, and frequencies adhered to Hardy-Weinberg equilibrium.

Table 2. Adverse Drug Events in 205 genotyped patients *

	Frequency (%) at 3 months	Frequency (%)at 6 months
Skin and mucosa disorders	12 (5.9)	17 (8.5)
Pneumonitis	0	0
Elevated liver enzyme levels	11 (5.4)	18 (9)
Gastrointestinal †	17 (8.3)	26 (13.0)
Overall adverse drug events	44 (21.5)	68 (34)

* Values for overall adverse drug events are the number of patients experiencing ≥ 1 event; values for the individual types of event are the number of events.
† General wellbeing, nausea, vomiting, diarrhea, constipation. Association of MTX efficacy and genetic polymorphisms

Association of MTX efficacy and genetic polymorphisms

MTHFR 1298AA was associated with response as defined by ΔDAS > 1.2. For MTHFR 677C>T, the CC genotype was associated with response as defined by ΔDAS > 0.6 at 6 months of treatment. After adjustment for multiple comparisons, the results for MTHFR 1298 remained significant (P = 0.042), whereas the results for MTHFR 677 did not (P = 0.132) (Table 3). In multivariate analyses, only the genotypes were found to contribute significantly to MTX efficacy as defined by ΔDAS > 1.2 (in analyses of MTHFR 1298, OR for lower baseline DAS score 1.48, 95% CI 1.00–2.20 and OR for RF positivity 0.56, 95% CI 0.27–1.16; in analyses of MTHFR 677, OR for lower baseline DAS score 1.63, 95% CI 0.95–2.79 and OR for RF positivity 0.69, 95% CI 0.25–1.89).

The RFC and DHFR SNPs were not associated with DAS improvement, and there was no association between response based on DAS ≤ 2.4 at 3 or 6 months and the individual genotypes (data not shown).

Table 3. Association of SNPs with MTX efficacy*

Group, improvement criterion	OR (95% CI)†	P	Adjusted P ‡
MTHFR 1298AA vs CA + CC, ΔDAS > 1.2	2.30 (1.18-4.41)	0.014	0.042
MTHFR 677CC vs CT + TT, ΔDAS > 0.6	2.73 (1.03-7.26)	0.044	0.132

* Reduced folate carrier (RFC) 80G>A, dihydrofolate reductase (DHFR) –473G>A and DHFR 35289 G>A were not found to be associated with efficacy. SNP = single nucleotide polymorphism, 95% CI = 95% confidence interval, MTHFR = methylene tetrahydrofolate reductase, MTX = methotrexate.
† Odds ratios (ORs) were corrected for age, sex, Disease activity Score in 44 joints (DAS) and rheumatoid factor positivity at baseline.
‡ Bonferroni correction.

The numbers of patients achieving DAS ≤ 2.4 response and good clinical improvement, by genotype for MTHFR, are presented in Tables 4 and 5. In general, the subgroup of patients still treated with 15 mg MTX/week at 6 months showed higher response percentages based on ΔDAS than those treated with 25 mg MTX/week (Table 5). The reason for this may be that the patients with early good response based on DAS ≤ 2.4 at 3 months, and who continued 15 mg/week MTX according to the protocol, had sustained and increased DAS response at 6 months.

Table 4. Number (%) of patients achieving DAS ≤ 2.4 response, by genotype for MTHFR*

Population (no.[%] of total)	3 months	6 months
MTHFR 677		
CC (93 [45])	18 (19)	39 (42)
CT (94 [46])	22 (23)	39 (41)
TT (18 [9])	4 (22)	9 (50)
MTHFR 1298		
AA (84 [41])	18 (21)	41 (49)
AC (96 [47])	22 (23)	36 (38)
CC (23 [11])	4 (17)	10 (43)

* DAS = Disease activity Score in 44 joints; MTHFR = methylenetetrahydrofolate reductase.

In addition, the effect of the MTHFR 1298A in relation to the 677C haplotype was assessed. The calculated D' value between the 2 SNPs was 0.91, and the r^2 was 0.20. For moderate clinical improvement, a significant association with the number of haplotype copies was found, and data suggested an association between good clinical improvement and the number of haplotype copies.

Of the patients with 2 copies of the haplotype (n = 26), 76.9% showed good clinical improvement at 6 months, compared with 65.8% with 1 copy of the haplotype (n = 76) and 61.9% with no copies (n = 84) (*P* not significant [NS]). After 6 months, 100% of patients carrying both MTHFR 677CC and 1298AA showed moderate clinical improvement, compared with 88.2% and 79.8% of patients with 1 and no copies, respectively ($P = 0.027$). In the multivariate regression analysis, the number of copies of the haplotype showed an increased OR with moderate improvement at 6 months (OR 3.0, 95% CI 1.4–6.4, adjusted $P = 0.021$). Moreover, the carriers of 2 copies of the haplotype showed more response, as defined by DAS ≤ 2.4 at 6 months (50%), when compared with carriers of MTHFR 677T allele and MTHFR 1298C allele (46.3%) at 6 months of therapy (NS).

Table 5. Number (%) of patients achieving DAS improvement criteria, by genotype for MTHFR *

	3 months	6 months, MTX 15 mg/week	6 months, MTX 25 mg/week
ΔDAS > 0.6			
MTHFR 677CC	63 (69)	22/25 (88)	51/56 (91)
MTHFR 677CT	65 (71)	24/27 (89)	47/61 (77)
MTHFR 677TT	12 (67)	3/3 (100)	9/14 (64)
ΔDAS > 1.2			
MTHFR 1298AA	33 (39)	17/19 (90)	42/61 (69)
MTHFR 1298AC	44 (48)	18/30 (60)	28/54 (52)
MTHFR 1298CC	10 (44)	4/6 (67)	9/14 (64)

* DAS = Disease Activity Score in 44 joints; MTHFR = methylene tetrahydrofolate reductase; MTX = methotrexate

Association of MTX toxicity and genetic polymorphisms

In the overall population, the prevalence of ADEs among patients taking 25 mg/week MTX was not significantly different from that among patients taking 15 mg/week (data not shown). In the regression analysis, MTHFR 1298AC + CC was associated with an increased number of overall ADEs compared with the MTHFR 1298AA genotype. When ADEs were specified, the data showed that the occurrence of gastrointestinal ADEs during the first 6 months of treatment contributed most (although this was not significant after Bonferroni correction) (Table 6).

For MTHFR 1298, 45% of the patients with the AC genotype (n = 42) experienced ADEs within 6 months, of which 42.9% (n = 18) were gastrointestinal ADEs. For the homozygous CC genotype this was 33.3% (n = 7), of which 28.6% were

gastrointestinal (n = 2). Of MTHFR 1298AA–genotype patients, 22.9% experienced ADEs (n = 19), with 31.6% being gastrointestinal (n = 6).

Our data suggested that the occurrence of hepatitis was associated with G carriers for DHFR -473G>A and 35289A>G. For DHFR 35289G>A, 12 patients with the GG genotype (12%), 6 patients with the GA genotype (4.8%), and none of the AA- genotyped patients experienced hepatitis ($P > 0.05$). For DHFR -473G>A, hepatitis occurred in 13 patients (12.6%) with the GG genotype, 3 (7.6%) with the GA genotype, and none with the AA genotype ($P > 0.05$). Among patients with the GG genotype for both DHFR SNPs (n = 38), hepatitis occurred in 21% (n = 8). Neither the single GG genotype nor the combination of the DHFR -473GG and 35289GG genotypes was associated with elevated liver enzyme levels at 6 months (OR 2.25, $P = 0.375$ for DHFR 35289GG; OR 2.60, $P = 0.471$ for DHFR -473GG; OR 3.33, $P = 0.066$ for the combination).

No associations were found between MTHFR 677C>T or RFC 80G>A and the occurrence of ADEs. Repeating analyses for toxicity and efficacy in the subcohort of white patients yielded similar results (data not shown).

Table 6. Association of SNPs with MTX toxicity in the MTHFR 1298AC + CC vs the AA group *

Adverse drug events	OR (95% CI) †	*P*	Adjusted *P* ‡
All at 3 months	2.55 (1.20-5.41)	0.015	0.045
All at 6 months	2.50 (1.32-4.72)	0.005	0.015
GI at 3 months	2.54 (0.79-8.13)	0.118	0.354
GI at 6 months	2.78 (1.05-7.30)	0.039	0.117

* MTHFR 677C>T, RFC 80G>A, DHFR –473G>A and DHFR 35289G>A were not found to be associated with toxicity. There were no other associations between defined adverse drug events and SNPs in the folate pathway. GI = gastrointestinal (See Table 3 for other definitions).
† All ORs were corrected for age and sex.
‡ Bonferroni correction.

Association of MTX efficacy combined with toxicity and genetic polymorphisms

In order to investigate whether the association of ADEs with MTHFR 1298A>C was related to response, toxicity regression analyses at 3 and 6 months were carried out in the subgroup of patients achieving ΔDAS > 1.2. In general, for all patients achieving ΔDAS > 1.2 at 3 and 6 months, there were no associations with overall ADEs or with any specific category of ADEs. At 6 months of follow-up, the risk for overall ADEs was slightly higher for responders with MTHFR 1298AC + CC (OR 2.72, 95% CI 1.14–6.45). More specifically, 63.3% of the patients with the MTHFR 1298AA genotype (n = 51) showed good clinical improvement at 6 months without ADEs, whereas for MTHFR 1298 C allele carriers (n = 40) this was 38.8%. However, 9.9% of the patients with the MTHFR 1298AA genotype (n = 9) did not show good clinical improvement but experienced an ADE, whereas for C allele carriers (n = 19) this was 18.5%.

DISCUSSION

This study shows that the MTHFR 1298AA and 677CC genotypes are associated with DAS improvement within the first 6 months of MTX treatment, whereas the MTHFR 1298C allele is associated with MTX toxicity. No associations with efficacy and toxicity were found for the individual SNPs RFC80G>A, DHFR -473G>A, and DHFR 38289G>A.

Although the functionality of MTHFR 677C>T and 1298A>C has not fully been explored, it has been shown that the effects of the mutant alleles lead to higher thermolability and a decrease in enzyme activity. The effects of RFC80 alleles have not been described, although it has been reported that RFC80AA-genotype patients have higher plasma folate and MTX levels (35,36), and higher red blood cell MTX polyglutamate levels (37). Recently, an association was suggested for a composite pharmacogenetic index, including the RFC80G>A SNP, with MTX efficacy (24,25). We were unable to detect associations of RFC80G>A with MTX efficacy and toxicity. Thus, the clinical implication of the single-polymorphism RFC80G>A in RA remains unclear. In other clinical studies with RA patients, the MTHFR 677T allele showed an association with toxicity (20,22) and the MTHFR 1298CC genotype was found to be inversely associated with ADEs (23), while no relationship between efficacy and MTHFR 677T allele carriers was shown (20).

The differences in our results compared with those reported by Urano and colleagues (22), who found an association between the MTHFR 677T allele and overall toxicity and between the MTHFR 1298C allele and lower MTX dosage, may be at least partly attributable to the selected population and study design. Urano and colleagues retrospectively studied a Japanese RA population (n = 106) selected from an outpatient clinic. Unlike the patients in our randomized controlled study, these patients had persistent RA, were previously treated with other DMARDs, and, in general, received lower MTX dosages (up to 12.5 mg/wk) without a standardized protocol for dosage adjustments. Only 2 patients received concomitant folic acid.

Berkun and colleagues (23) identified an association of MTHFR 1298CC with a reduction of MTX ADEs in a cross-sectional RA population (n = 93). This differs from our results, and may reflect the heterogeneity in outcome measures for ADEs and the differences in MTHFR 1298CC genotype frequency. Our study showed genotype frequencies with a distribution resembling that described in previous publications and adhering to Hardy-Weinberg equilibrium.

Consistent with our findings, Van Ede and colleagues (20) found no association between the MTHFR 677T allele and overall hepatotoxicity, but they did note an association with hepatotoxicity-related discontinuation of MTX. In the present study, all patients received concomitant folic acid, and MTX was discontinued due to ADEs in only 11 patients (5.4%). In the cohort of Van Ede and colleagues, 24% of the patients (n = 57) discontinued MTX, partly due to the design of the study, in which only two-thirds of the patients received folic or folinic acid. Regarding efficacy, Van Ede and colleagues found no significant difference in the average change in DAS in MTHFR

677T allele carriers versus noncarriers. It has been shown that disease duration and previous treatment influence MTX outcome (5,6), and their cohort included patients with persistent RA who had previously taken other DMARDs. We believe our results are most applicable to patients with early RA who have not received DMARDs.

In general, the replication of results in genetic association studies is complicated, and comparisons between studies are difficult. The best evidence of an association remains the replication of this association with the same genotype, the same phenotype, and the identical direction of effect in an independent but identical population. Most genetic association studies do not have sufficient power given the limited number of patients with the homozygous mutant genotype. For studying associations with toxicity this problem is even more common given the low incidence of this outcome. In our analyses, adjustment of *P* values using the Bonferroni correction minimized false-positive results and reduced the statistical power of the association of MTHFR with efficacy and toxicity of MTX. However, this approach is conservative and increases the chance of a Type II error (38,39); therefore, both the adjusted and unadjusted *P* values are presented.

Notably, the identified confounders RF and DAS at baseline were not associated with MTX efficacy in the multivariate analysis including MTHFR genotypes. The differences in clinical characteristics at baseline between responders and nonresponders showed no association and did not affect the MTHFR genotype association in an additional multivariate efficacy analysis (data not shown). This emphasizes the robustness of our results, because the MTHFR genotype is the only strong predictor of MTX efficacy.

Although our study was not primarily designed to reveal associations between SNPs and MTX efficacy and toxicity, our approach had several advantages compared with other genetic association studies. This study included 205 patients treated with standardized dosages of MTX and folic acid with fixed treatment duration. The protocol was designed to compare RA treatments, thus offering clear response and toxicity measures in DMARD-naive patients.

One of the difficulties in evaluating response to treatment in RA is the need for consensus in efficacy and toxicity measures. There is also a difference of opinion on matters such as the time period and trial methodology needed (40–44). Ideally for clinical purposes, there are absolute cutoff values to classify patients as responders or nonresponders and provide treatment guidelines. However, in clinical practice any change in toxicity and efficacy measures is a motive for physicians to adjust treatment (4,45).

Because "good response," not "good improvement," is the ultimate clinical goal of treatment in RA, the BeSt study aimed for achievement of a DAS score of ≤ 2.4 in all patients. Therefore, treatment was adjusted every 3 months if DAS $\leq$ 2.4 was not achieved. This did not allow for analysis of whether patients with a certain genotype would achieve clinical response (as defined by ΔDAS) with a longer treatment duration (slow responders), increased dosage of MTX (poor responders), or a combination of these factors.

Our data suggest that there are differences by genotype in MTX response with increased dosage and longer treatment duration (Table 5). For instance, patients with MTHFR 1298AA show a higher percentage of good clinical improvement at 6 months in the 15 mg/wk group as well in the 25 mg/wk group when compared with C allele carriers. In contrast, patients with the MTHFR 1298AC genotype do not seem to improve much with increased dosage (25 mg/wk group) or with a longer treatment period (15 mg/wk group), whereas the clinical improvement of MTHFR 1298CC–genotype patients seems attributable to increased MTX dosage and treatment duration. For MTHFR 677C>T, data suggest that T allele carriers show relatively less clinical improvement upon increased MTX dosage (Table 5). These observations indicate that ascertainment of MTHFR genotype could be useful in establishing the initial MTX dosage to more rapidly reduce disease activity in patients with early RA.

In conclusion, this study shows that SNPs in MTHFR are predictive of response to and safety of MTX treatment. Our results reveal associations of the MTHFR 1298AA genotype and MTHFR 677CC with response, and the MTHFR 1298C allele with toxicity, in early RA.

Role of the funding sources

Supported by the Foundations Nuts Ohra, Amsterdam, The Netherlands, and by De Drie Lichten, Leiden, The Netherlands. The BeSt study was funded by a grant of the Dutch College of Health Insurances (College voor zorgverzekeringen) with additional funding provided by Schering-Plough, B.V. and Centocor, Inc. The authors were responsible for the study design, the collection, analysis and interpretation of all data, the writing of this article and the decision to publish.

REFERENCES

1. Jones G, Halbert J, Crotty M, Shanahan EM, Batterham M, Ahern M. The effect of treatment on radiological progression in rheumatoid arthritis: a systematic review of randomized placebo- controlled trials [review]. Rheumatology (Oxford) 2003;42:6–13.
2. Pincus T, Ferraccioli G, Sokka T, Larsen A, Rau R, Kushner I, et al. Evidence from clinical trials and long-term observational studies that disease-modifying anti-rheumatic drugs slow radiographic progression in rheumatoid arthritis: updating a 1983 review [review]. Rheumatology (Oxford) 2002;41:1346–56.
3. Aletaha D, Smolen JS. The rheumatoid arthritis patient in the clinic: comparing more than 1,300 consecutive DMARD courses. Rheumatology (Oxford) 2002;41:1367–74.
4. Maetzel A, Wong A, Strand V, Tugwell P, Wells G, Bombardier C. Meta-analysis of treatment termination rates among rheumatoid arthritis patients receiving disease-modifying anti-rheumatic drugs. Rheumatology (Oxford) 2000;39:975–81.
5. Hoekstra M, van Ede AE, Haagsma CJ, van de Laar MA, Huizinga TW, Kruijsen MW, et al. Factors associated with toxicity, final dose, and efficacy of methotrexate in patients with rheumatoid arthritis. Ann Rheum Dis 2003;62:423–6.
6. Anderson JJ, Wells G, Verhoeven AC, Felson DT. Factors predicting response to treatment in rheumatoid arthritis: the importance of disease duration. Arthritis Rheum 2000;43:22–9.
7. Van Ede AE, Laan RF, Rood MJ, Huizinga TW, van de Laar MA, van Denderen CJ, et al. Effect of folic or folinic acid supplementation on the toxicity and efficacy of methotrexate in rheumatoid arthritis: a forty-eight–week, multicenter, randomized, double- blind, placebo-controlled study. Arthritis Rheum 2001;44:1515–24.
8. Gossec L, Dougados M, Goupille P, Cantagrel A, Sibilia J, Meyer O, et al. Prognostic factors for remission in early rheumatoid arthritis: a multiparameter prospective study. Ann Rheum Dis 2004;63:675–80.
9. Scott DL. Evidence for early disease-modifying drugs in rheumatoid arthritis. Arthritis Res Ther 2004;6:15–8.
10. Matteson EL, Weyand CM, Fulbright JW, Christianson TJ, Mc- Clelland RL, Goronzy JJ. How aggressive should initial therapy for rheumatoid arthritis be? Factors associated with response to 'non-aggressive' DMARD treatment and perspective from a 2-yr open label trial. Rheumatology (Oxford) 2004;43:619–25.
11. Verstappen SM, Jacobs JW, Bijlsma JW, Heurkens AH, van Booma-Frankfort C, Borg EJ, et al. Five-year followup of rheumatoid arthritis patients after early treatment with disease-modifying antirheumatic drugs versus treatment according to the pyramid approach in the first year. Arthritis Rheum 2003;48: 1797–807.
12. Nell VP, Machold KP, Eberl G, Stamm TA, Uffmann M, Smolen JS. Benefit of very early referral and very early therapy with disease-modifying anti-rheumatic drugs in patients with early rheumatoid arthritis. Rheumatology (Oxford) 2004;43:906–14.
13. Van Aken J, Lard LR, le Cessie S, Hazes JM, Breedveld FC, Huizinga TW. Radiological outcome after four years of early versus delayed treatment strategy in patients with recent onset rheumatoid arthritis. Ann Rheum Dis 2004;63:274–9.

14. Ranganathan P, Eisen S, Yokoyama WM, McLeod HL. Will pharmacogenetics allow better prediction of methotrexate toxicity and efficacy in patients with rheumatoid arthritis? [review]. Ann Rheum Dis 2003;62:4–9.
15. Ulrich CM, Yasui Y, Storb R, Schubert MM, Wagner JL, Bigler J, et al. Pharmacogenetics of methotrexate: toxicity among marrow transplantation patients varies with the methylenetetrahydrofolate reductase C677T polymorphism. Blood 2001;98:231– 4.
16. Siva C, Yokoyama WM, McLeod HL. Pharmacogenetics in rheumatology: the prospects and limitations of an emerging field [review]. Rheumatology (Oxford) 2002;41:1273–9.
17. Ranganathan P, Culverhouse R, Marsh S, Ahluwalia R, Shannon WD, Eisen S, et al. Single nucleotide polymorphism profiling across the methotrexate pathway in normal subjects and patients with rheumatoid arthritis. Pharmacogenomics 2004;5:559–69.
18. Kremer JM. Toward a better understanding of methotrexate [review]. Arthritis Rheum 2004;50:1370–82
19. Cronstein BN. The mechanism of action of methotrexate [review]. Rheum Dis Clin North Am 1997;23:739–55.
20. Van Ede AE, Laan RF, Blom HJ, Huizinga TW, Haagsma CJ,Giesendorf BA, et al. The C677T mutation in the methylenetetrahydrofolate reductase gene: a genetic risk factor for methotrexate-related elevation of liver enzymes in rheumatoid arthritis patients. Arthritis Rheum 2001;44:2525–30.
21. Haagsma CJ, Blom HJ, van Riel PL, van 't Hof MA, Giesendorf BA, van Oppenraaij-Emmerzaal D, et al. Influence of sulphasalazine, methotrexate, and the combination of both on plasma homocysteine concentrations in patients with rheumatoid arthritis. Ann Rheum Dis 1999;58:79–84.
22. Urano W, Taniguchi A, Yamanaka H, Tanaka E, Nakajima H, Matsuda Y, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene were associated with both the efficacy and the toxicity of methotrexate used for the treatment of rheumatoid arthritis, as evidenced by single locus and haplotype analyses. Pharmacogenetics 2002;12:183–90.
23. Berkun Y, Levartovsky D, Rubinow A, Orbach H, Aamar S, Grenader T, et al. Methotrexate related adverse effects in patients with rheumatoid arthritis are associated with the A1298C polymorphism of the MTHFR gene. Ann Rheum Dis 2004;63:1227–31.
24. Dervieux T, Furst D, Lein DO, Capps R, Smith K, Walsh M, et al. Polyglutamation of methotrexate with common polymorphisms in reduced folate carrier, aminoimidazole carboxamide ribonucleotide transformylase, and thymidylate synthase are associated with methotrexate effects in rheumatoid arthritis. Arthritis Rheum 2004;50:2766–74.
25. Dervieux T, Furst D, Lein DO, Capps R, Smith K, Caldwell J, et al. Pharmacogenetic and metabolite measurements are associated with clinical status in patients with rheumatoid arthritis treated with methotrexate: results of a multicentered cross sectional observational study. Ann Rheum Dis 2005;64:1180–5.
26. Kumagai K, Hiyama K, Oyama T, Maeda H, Kohno N. Polymorphisms in the thymidylate synthase and methylenetetrahydrofolate reductase genes and sensitivity to the low-dose methotrexate therapy in patients with rheumatoid arthritis. Int J Mol Med 2003;11:593–600.

27. Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Allaart CF, van Zeben D, Kerstens PJ, Hazes JM, et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): a randomized, controlled trial. Arthritis Rheum 2005;52:3381–90.
28. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
29. Van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis: comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. Arthritis Rheum 1996;39:34– 40.
30. Van der Heijde DM, van 't Hof M, van Riel PL, van de Putte LB. Development of a disease activity score based on judgment in clinical practice by rheumatologists. J Rheumatol 1993;20:579–81.
31. Jurinke C, van den Boom D, Cantor CR, Koster H. The use of MassARRAY technology for high throughput genotyping [review]. Adv Biochem Eng Biotechnol 2002;77:57–74.
32. Alderborn A, Kristofferson A, Hammerling U. Determination of single-nucleotide polymorphisms by real-time pyrophosphate DNA sequencing. Genome Res 2000;10:1249–58.
33. Van der Heijde DM, van Riel PL, Nuver-Zwart IH, Gribnau FW, van de Putte LB. Effects of hydroxychloroquine and sulphasalazine on progression of joint damage in rheumatoid arthritis. Lancet 1989;1:1036–8.
34. Ritchie DM, Boyle JA, McInnes JM, Jasani MK, Dalakos TG, Grieveson P, et al. Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. QJM 1968;37:393– 406.
35. Laverdiere C, Chiasson S, Costea I, Moghrabi A, Krajinovic M. Polymorphism G80A in the reduced folate carrier gene and its relationship to methotrexate plasma levels and outcome of childhood acute lymphoblastic leukemia. Blood 2002;100:3832– 4.
36. Chango A, Emery-Fillon N, de Courcy GP, Lambert D, Pfister M, Rosenblatt DS, et al. A polymorphism (80G>A) in the reduced folate carrier gene and its associations with folate status and homocysteinemia. Mol Genet Metab 2000;70:310–5.
37. Dervieux T, Kremer J, Lein DO, Capps R, Barham R., Meyer G, et al. Contribution of common polymorphisms in reduced folate carrier and gamma-glutamylhydrolase to methotrexate polyglutamate levels in patients with rheumatoid arthritis. Pharmacogenetics 2004;14:733–9.
38. Newton-Cheh C, Hirschhorn JN. Genetic association studies of complex traits: design and analysis issues [review]. Mutat Res 2005;573:54–69.
39. Huizinga TW, Pisetsky DS, Kimberly RP. Associations, populations, and the truth: recommendations for genetic association studies in Arthritis & Rheumatism [review]. Arthritis Rheum 2004;50:2066–71.
40. Pincus T. Assessment of long-term outcomes of rheumatoid arthritis: how choices of measures and study designs may lead to apparently different conclusions [review]. Rheum Dis Clin North Am 1995;21:619–54.

41. Albert DA, Huang G, Dubrow G, Brensinger CM, Berlin JA, Williams HJ. Criteria for improvement in rheumatoid arthritis: alternatives to the American College of Rheumatology. J Rheumatol 2004;31:856–66.
42. Pincus T, Stein CM. ACR 20: clinical or statistical significance? Arthritis Rheum 1999;42:1572–6.
43. Van Riel PL, van Gestel AM, van de Putte LB. Development and validation of response criteria in rheumatoid arthritis: steps towards an international consensus on prognostic markers. Br J Rheumatol 1996;35 Suppl 2:4–7.
44. Felson DT. Whither the ACR20? [review]. J Rheumatol 2004;31:835–7.
45. Hoekstra M, van de Laar MA, Bernelot Moens HJ, Kruijsen MW, Haagsma CJ. Longterm observational study of methotrexate use in a Dutch cohort of 1022 patients with rheumatoid arthritis. J Rheumatol 2003;30:2325–9.

Chapter 9

Progression of joint damage in early rheumatoid arthritis

Association with HLA DRB1, rheumatoid factor and anti-CCP antibodies (ACPA) for different treatment strategies

J. K. de Vries-Bouwstra
Y. P. M. Goekoop-Ruiterman
K. N. Verpoort
G. M. T. Schreuder
J. A. P. M. Ewals
J. P. Terwiel
H. K. Ronday
P. J. S. M. Kerstens
R. E. M. Toes
R. R. P. de Vries
F. C. Breedveld
B. A. C. Dijkmans
T. W. J. Huizinga
C. F. Allaart

submitted

ABSTRACT

Objectives To determine the association of HLA DRB1, rheumatoid factor (RF), and anti-cyclic citrullinated peptide antibodies (ACPA) with joint damage progression in early rheumatoid arthritis (RA) treated according to different treatment strategies.

Methods The BeSt study was a randomized trial, with 508 early RA patients, that compared 4 targeted (DAS ≤ 2.4) treatment strategies: (1) sequential monotherapy, (2) step-up combination therapy, and initial combination therapy with (3) methotrexate, sulphasalazine and prednisone, or (4) methotrexate and infliximab. Multivariate logistic regression was used to predict progressive disease (increase of Sharp-van der Heijde-score over 2 years > Smallest Detectable Change = 4.6) given shared epitope (SE), DERAA, RF and ACPA with correction for other baseline characteristics.

Results Progressive disease could not be predicted by presence of SE (odds ratio [OR; 95% CI] 1.4 [0.4-5.0], 2.6 [0.8-8.7], 1.9 [0.5-7.4], 3.0 [0.7-13.0] for groups 1-4). DERAA-carriership did not protect against progressive disease (OR 0.4 [0.1-1.2], 1.4 [0.3-5.5], 0.9 [0.2-3.7], 0.9 [0.2-3.1] for groups 1-4). RF and ACPA did predict progressive disease in group 1 (OR RF: 4.7 [1.5-14.5], ACPA 12.6 [3.0-51.9]) but not in groups 2-4 (OR RF 1.5 [0.5-4.9], 1.0 [0.3-3.3], 1.4 [0.4-4.8]; ACPA 3.4 [0.8-14.2], 1.7 [0.5-5.4], 1.8 [0.5-6.8] for groups 2-4).

Conclusions The association of HLA DRB1 with joint damage progression was not found in RA patients treated early with tight control of DAS. RF and ACPA were only predictive of progressive disease in patients treated with sequential monotherapy, indicating that effective treatment neutralizes the detrimental effects of HLA DRB1 and autoantibodies on joint damage.

INTRODUCTION

The presence of certain human leukocyte antigen (HLA) class II alleles and autoantibodies, notably rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (ACPA), are known to be associated with susceptibility to and severity of rheumatoid arthritis (RA) (1,2).

Many studies have shown that the presence of the HLA DRB1 alleles *0101, *0102, *0401, *0404, *0405, *0408, *0410, *1001, or *1402 is associated with susceptibility to and severity of RA. These alleles all share an amino acid sequence in the third hypervariable region (HVR3, QKRAA/QRRAA/RRRAA, known as the shared epitope (SE) (2). Other studies have shown a negative association with progression to RA for some HLA DRB1 alleles (*0103, *0402, *1102, *1103, *1301, *1302, and *1304), which all contain the DERAA motif in the HVR3, the so-called RA-protection model (RAP) (3). Recently, it was shown that the presence of DERAA also protects against more severe radiographic progression in patients prone to more severe disease (4).

RF is found in the sera of 50% - 80% of RA patients (5). The presence of RF is not unique for RA patients but is consistently reported to be associated with more severely destructive RA (6). More recently, the presence of ACPA was shown to be predictive of and highly specific for RA (7). ACPAs are also associated with more destructive disease (1). Interestingly, HLA-SE alleles are only associated with RA in the presence of ACPA but not with ACPA negative disease (8).

In order to better prevent a severe and destructive disease course, treatment of RA can be initiated immediately after diagnosis, while combinations of antirheumatic drugs, including biologics, are used more often. Many of the studies demonstrating the association between autoantibodies and/or SE alleles with more severe progression of joint damage derived data from early arthritis inception cohorts including RA patients not treated according to current standards (9,10). Lard and colleagues showed that with early and aggressive treatment the association between the presence of SE and progression of joint damage disappears (11). In the BeSt study, a comparison of four intensive treatment strategies in patients with newly diagnosed RA, treatment was adjusted until a low disease activity (DAS < 2.4) was achieved. In all treatment groups, this resulted in a remarkable improvement in physical function and suppression of joint damage progression after 2 years of treatment (12). In this unique cohort of patients, we explored the influence of the four treatment strategies on the association between HLA DRB1, RF, ACPA, and joint destruction.

PATIENTS AND METHODS

Patients and treatment protocol

The BeSt study was a randomized, controlled multicenter trial of 508 patients with recent-onset RA (ACR 1987 criteria) who were allocated into one of four treatment strategy

groups. Patients fulfilled criteria for active disease with at least 6 out of 66 swollen joints, 6 out of 68 painful joints, and either an erythrocyte sedimentation rate (ESR) of at least 28 mm/hour (hr) or a score of at least 20 mm on a visual analog scale (VAS) for general well being by the patient. The median symptom duration at inclusion was 23 weeks (interquartile range 14-53), with a median period between diagnosis and inclusion, ie start of treatment, of 2 weeks (interquartile range 1-5). Prior treatment with disease-modifying antirheumatic drugs (DMARDs), except for antimalarials, was not allowed. All patients were randomly allocated to one of four treatment groups: sequential monotherapy (n = 126), step-up combination therapy (n = 121), initial combination therapy including a high-tapered dose prednisone (n = 133), and initial combination therapy including infliximab (n = 128). In all groups the goal was to achieve good clinical response as defined by the disease activity score (DAS). Every 3 months, the DAS, based on a 44 joint count, was calculated by a research nurse blinded to the treatment group. If the DAS was greater than 2.4, the treatment was adjusted according to the protocol of the particular treatment group. If the DAS was less than or equal to 2.4 for at least 6 months, the number and doses of drugs were tapered until only one drug remained at a maintenance dose. Further details concerning patient population, treatment protocol and primary outcomes have been published elsewhere (12).

Baseline characteristics

The following parameters were assessed in all patients at study entry: age, sex, current smoking status, duration of symptoms, time between diagnosis and study inclusion, swollen joint count (SJC), number of painful joints (Ritchie Articular Index), C-reactive proteine (CRP), ESR, VAS by the patient for disease activity, VAS by the patient for morning stiffness, VAS by the patient for pain, VAS by the patient for general well-being, DAS, radiographic damage score (see below), presence of erosions, and functional ability as measured with the health assessment questionnaire (HAQ).

HLA genotyping

HLA class II typing was performed in all patients who gave written informed consent for the sampling of DNA material (group 1 n = 113, group 2 n = 102, group 3 n = 115, and group 4 n = 111). HLA DRB1 (sub)-typing was performed by polymerase chain reaction, using specific primers and hybridization with sequence specific oligonucleotides. All patients carrying HLA DRB1 *0101, *0102, *0401, *0404, *0405, *0408, *0410, *1001, or *1402 were considered SE-positive; all patients carrying HLA-DRB1 *0103, *0402, *1102, *1103, *1301, *1302, and *1304 were considered DERAA-positive. Based on the (sub)-typing results, SE-carriership was determined in 417 patients (94% of typed patients) and DERAA-carriership was determined in 383 patients (87% of typed patients).

Autoantibodies

The presence of RF was measured at baseline by the laboratories of the centers participating in the BeSt study (n = 20). RF status was determined for each patient based on the validated cut-off value of the particular laboratory. At the time of enrollment, determination of ACPA was not commonly available in the participating centers and different commercial kits were used in the different laboratories (Euro-Diagnostica, Arnhem, The Netherlands; Axis Shield, via Orange Medical, Tilburg, The Netherlands). In 119 patients the presence of ACPA was determined using baseline sera. For 309 patients, the presence of ACPA was determined from serum samples obtained during follow-up. Recent evidence indicates that the presence or absence of ACPA is a stable characteristic (13). Therefore, all patients with ACPA status available were included in the analysis, in total 428 patients (84%) (group 1 n = 104 [83%], group 2 n = 104 [86%], group 3 n = 109 [82%], group 4 n = 111 [87%]).

Radiographic progression

Radiographs of both hands and feet at baseline and after 2 years were available for 455 patients (90%) (group 1 n = 111 [88%], group 2 n = 105 [87%], group 3 n = 123 [92%], group 4 n = 116 [91%]). Radiographic progression over 2 years was determined according to the Sharp-van der Heijde method by two observers who scored the anonymous radiographs of hands and feet independently, paired and in random order. The intra-observer coefficients were 0.90 and 0.91 and the inter-observer coefficient was 0.94. The mean score of the two observers was used for all analyses. Erosive disease was defined as a mean erosion score of at least 0.5. Progressive disease was defined as a change in the total Sharp-van der Heijde score over 2 years that was greater than the smallest detectable change, which was 4.64 (12).

Statistical analysis

Baseline characteristics among subgroups of patients were compared using ANOVA or Kruskal-Wallis tests where appropriate for comparisons of means and medians and chi-square tests for comparison of proportions. Logistic regression analysis was performed to evaluate whether progressive disease was significantly influenced by the presence of SE, DERAA, RF or ACPA in any of the treatment groups after correction for characteristics significantly associated with radiographic outcome and/or presence of SE, DERAA, RF, or ACPA.

RESULTS

Table 1. Baseline patient characteristics; median (interquartile range) for continuous variables and number (%) for discrete variables

	Sequential monotherapy	Step-up combination therapy	Initial combination therapy with prednisone	Initial combination therapy with infliximab
Age	54 (45-63)	54 (45-64)	55 (43-65)	54 (45-63)
Females	86 (68)	86 (71)	86 (65)	85 (66)
Duration of symptoms (weeks)	23 (14-54)	26 (14-56)	23 (15-52)	23 (13-46)
Time between diagnosis and inclusion (weeks)	2 (1-5)	2 (1-4)	2 (1-4)	3 (1-5)
HAQ	1.4 (1.0-1.9)	1.4 (0.9-1.9)	1.4 (1.0-2.0)	1.4 (0.9-1.8)
DAS	4.5 (3.9-5.2)	4.4 (3.9-5.1)	4.3 (3.8-5.0)	4.2 (3.7-4.9)
Sharp-van der Heijde score†	3.5 (1.5-9.5)	5.0 (1.5-8.1)	3.5 (1.5-8.5)	4.0 (1.5-8.5)
Erosive disease†	89 (72)	82 (70)	93 (71)	93 (73)
Rheumatoid factor positive	84 (67)	77 (64)	86 (65)	82 (64)
ACPA positive‡	68 (65)	61 (59)	60 (55)	72 (65)
Shared Epitope positive*	69 (64)	60 (62)	75 (69)	74 (71)
DERAA positive$^{\Delta}$	19 (19)	10 (11)	16 (16)	22 (23)
Smoking•	45 (37)	44 (39)	44 (35)	40 (32)

† n = 455; ‡ n = 428; * n = 417; $^{\Delta}$ n = 383; • n = 487

At baseline, disease characteristics for patients in the four groups were comparable (Table 1). In total, 67% were SE carriers and 17% were DERAA carriers, equally distributed among the treatment groups. Sixty-five percent of patients were positive for RF and 61% were positive for ACPA, also equally distributed among the four groups (Table 1).

Over 2 years of follow-up, radiographic progression was not significantly different between SE-positive patients and SE-negative patients (Figure 1A; median [interquartile range] group 1 SE- 1.0 [-0.5-5.0], SE+ 3.8 [0.1-11.6]; group 2 SE- 1.0 [0.0-3.0], SE+ 3.0 [0.5-10.0]; group 3 SE- 2.1 [0.5-4.8], SE+ 1.0 [0.0-5.5]; group 4 SE- 0.0 [-0.5-1.1], SE+ 1.5 [0.0-4.0]; $P > 0.05$) and between DERAA positive and DERAA negative patients (median [interquartile range] group 1 DERAA- 3.3 [0.0-10.9], DERAA+ 1.5 [-0.5-5.0]; group 2 DERAA- 2.0 [0.5-9.5], DERAA+ 3.8 [0.8-10.3]; group 3 DERAA- 1.0 [0.0-3.0], DERAA+ 0.8 [0.0-3.9]; group 4 DERAA- 1.5 [0.0-4.0], DERAA+ 1.0 [0.0-4.0]; $P > 0.05$ for all comparisons; probability plots not shown because of small number of DERAA carriers) irrespective of the treatment group. Of patients treated with sequential monotherapy and step-up combination therapy, radiographic progression scores were significantly higher in RF-positive patients compared with RF-negative patients and also in ACPA-positive patients compared with ACPA-negative patients (Figure 1B and 1C; median [interquartile range] group 1 RF- 0.0 [-0.5 - 3.9], RF+ 4.8 [0.5-20.0], ACPA- 0.0 [-0.5-2.1], ACPA+ 5.0 [0.5 - 21.5]; group 2 RF- 1.0 [0.0-4.0], RF+ 2.5 [0.5-11.1], ACPA- 0.8 [0.0-3.9], ACPA+ 3.0 [1.0-11.5]; $P < 0.05$ for all comparisons). For patients treated with initial combination therapy including prednisone, radiographic progression scores were comparable between RF-positive and RF-negative patients and between ACPA-positive and ACPA-negative patients (Figure 1B

Figure 1. Radiographic progression (Sharp-Van der Heijde score; probability plots) over 2 years for subsets of patients defined according to presence or absence of Shared Epitope (SE; 1A), Rheumatoid factor (RF; 1B) and anti-CCP (ACPA; 1C) in the four treatment groups

Figure 1A. Radiographic progression (y-ax) for SE-positive and SE-negative patients

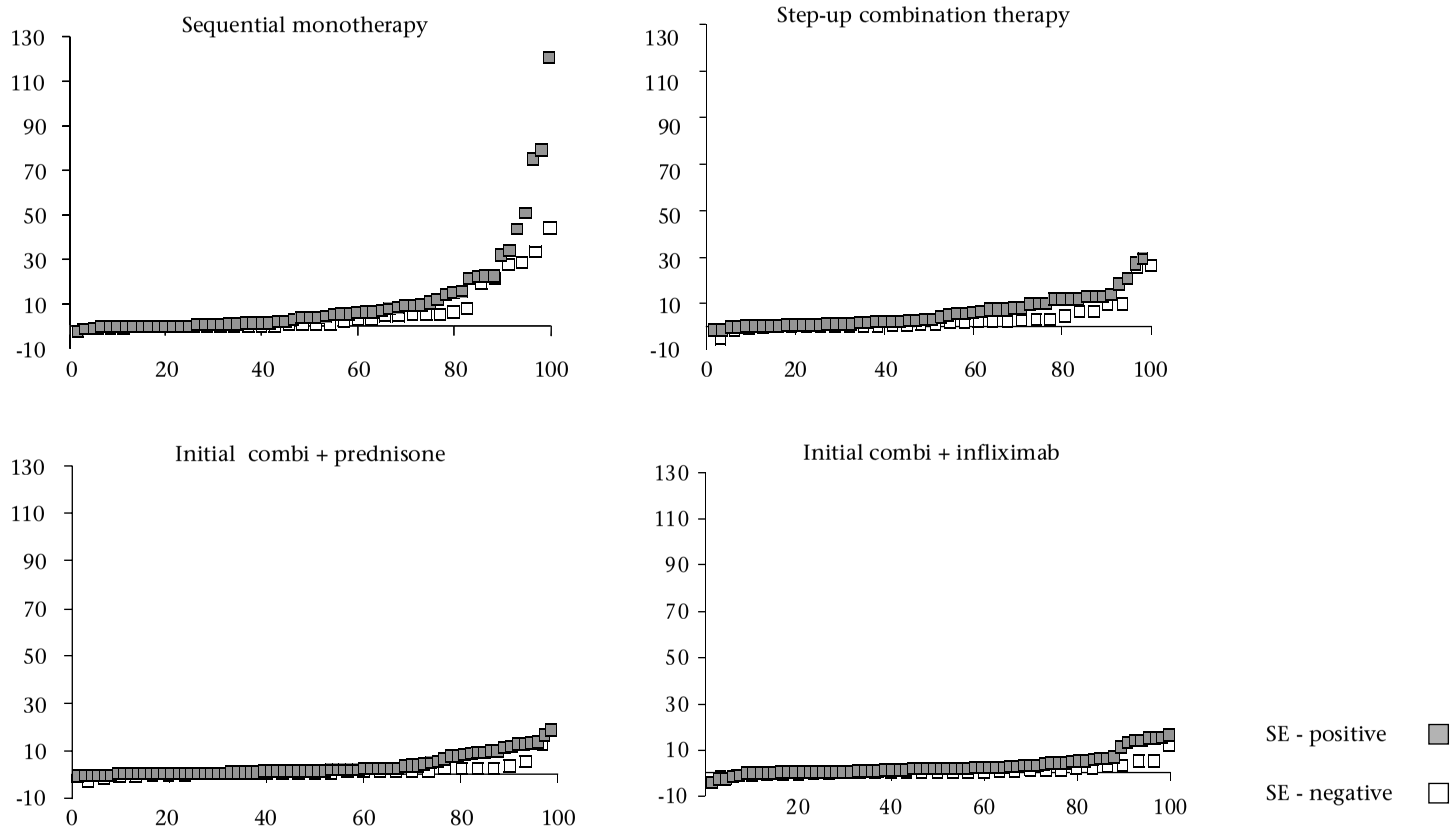


Figure 1B. Radiographic progression (y-ax) for RF-positive and RF-negative patients

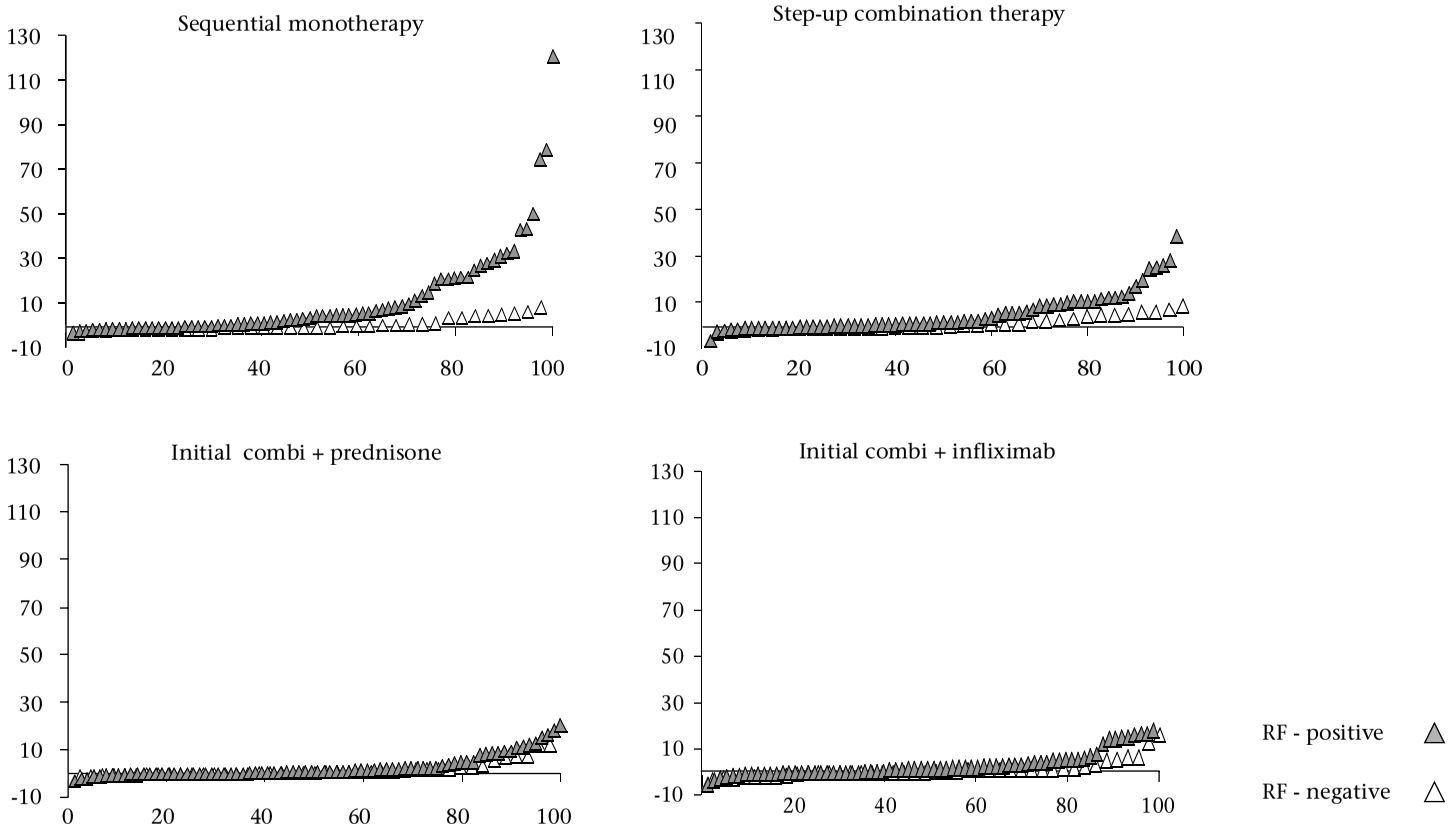
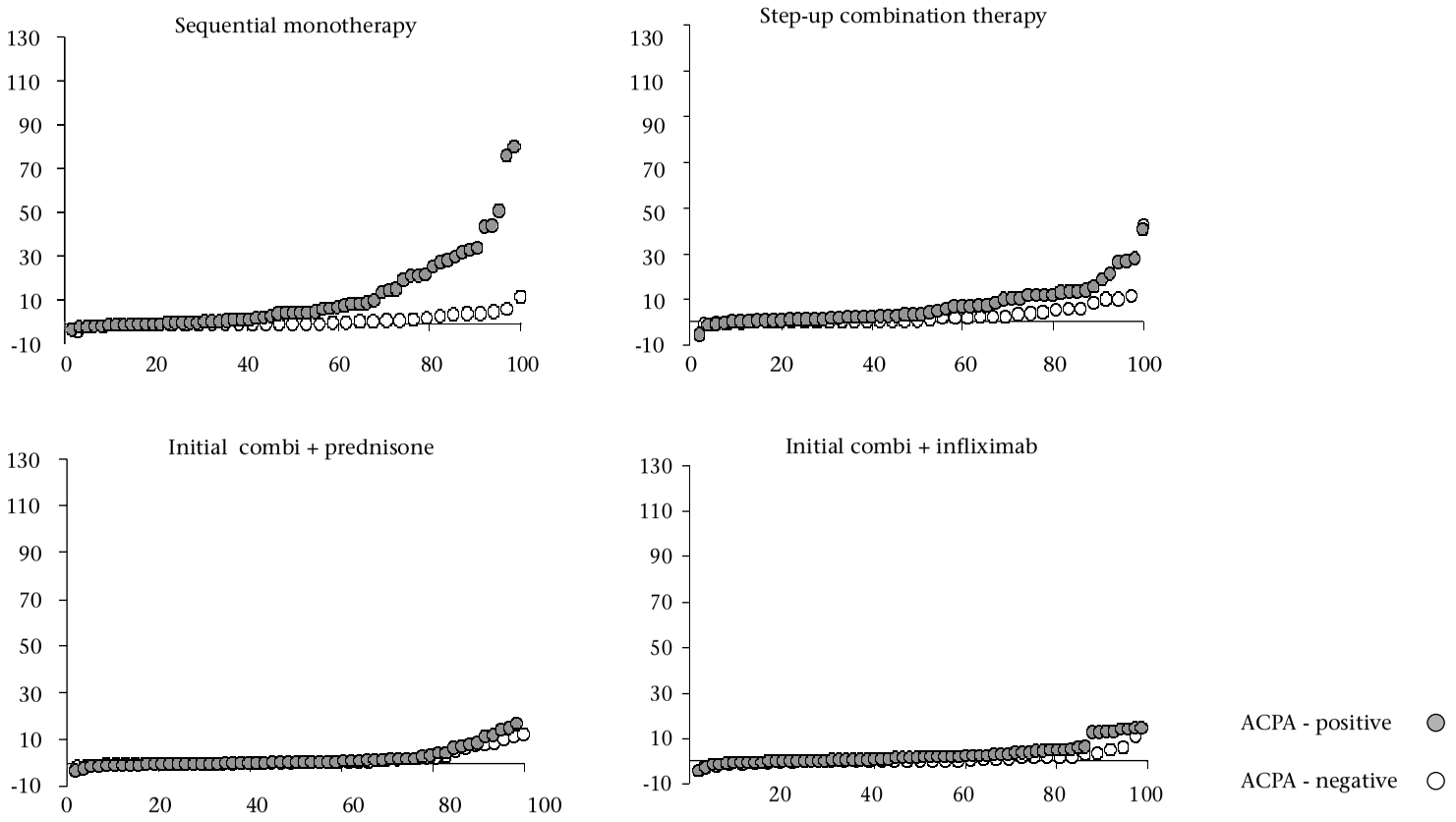


Figure 1C. Radiographic progression (y-ax) for ACPA-positive and ACPA-negative patients



and 1C; median [interquartile range] group 3 RF- 0.8 [0.0-2.0], RF+ 1.0 [0.0-3.0], ACPA- 1.0 [0.0-2.5], ACPA+ 1.0 [0.0-2.8]; $P > 0.05$ for both comparisons). For patients treated with initial combination therapy including infliximab, the differences in radiographic progression scores between RF-positive patients and RF-negative and between ACPA-positive and ACPA-negative were statistically significant although probability plots showed similar curves for RF-positive and RF-negative patient s and for ACPA-positive and ACPA-negative patients (Figure 1B and 1C; median [interquartile range] group 4 RF- 0.5 [0.0-1.5], RF+ 1.5 [0.0-4.3], ACPA- 0.0 [0.0-1.5], ACPA+ 2.0 [0.0-4.5], $P < 0.05$ for both comparisons).

SE-carriership (corrected for ACPA, CRP and erosions at baseline) did not predict progressive disease in any of the treatment groups. DERAA-carriership (corrected for age and erosions at baseline) did not predict protection against progressive disease. The presence of RF (corrected for gender, smoking status, SE, DAS, and erosions at baseline) and the presence of ACPA (corrected for gender, smoking status, SE, DAS, and erosions at baseline) were predictive of progressive disease in patients treated with sequential monotherapy but not in the other treatment groups (Table 2).

Table 2. Risk (odds ratios [95% confidence intervals]) for progressive disease with presence of shared epitope, DERAA, Rheumatoid factor, or ACPA

	Sequential monotherapy	Step-up combination therapy	Initial combination therapy with prednisone	Initial combination therapy with infliximab
Shared epitope	1.4 (0.4-5.0)	2.6 (0.8-8.7)	1.9 (0.5-7.4)	3.0 (0.7-13.0)
DERAA	0.4 (0.1-1.2)	1.4 (0.3-5.5)	0.9 (0.2-3.7)	0.9 (0.2-3.1)
Rheumatoid factor	4.7 (1.5-14.5)	1.5 (0.5-4.9)	1.0 (0.3-3.3)	1.4 (0.4-4.8)
ACPA	12.6 (3.0-51.9)	3.4 (0.8-14.2)	1.7 (0.5-5.4)	1.8 (0.5-6.8)

All odds ratios (OR) corrected for presence of erosions at baseline. OR for shared epitope (SE) corrected for ACPA and C-reactive proteine at baseline; OR for DERAA corrected for age; OR for rheumatoid factor corrected for gender, smoking status, SE and DAS; OR for anti CCP corrected for gender, smoking status, SE and DAS.

DISCUSSION

This analysis of the BeSt study showed no significant association between HLA DRB1 and radiographic progression in patients with early, active RA who were treated with frequent therapy adjustments to achieve a low disease activity. These data are consistent with the earlier observations of Lard and colleagues, who found no association between SE and radiographic progression with early, intensive treatment (11). An association between RF and ACPA positive status and radiological damage progression was still observed, notably in patients treated with sequential monotherapy.

According to the SE hypothesis, the SE motif, as part of the peptide binding groove of the HLA DR molecule, plays a role in the presentation of arthritogenic peptides to T cells, possibly inducing autoreactive T cells. More recently, it has been suggested that the

association of SE with more severe progression of joint damage may be partly explained by the presence of ACPA (8). Although several hypotheses have been formulated to explain the contribution of biological pathways to disease induction and progression, current data suggest that with early and aggressive intervention a self-perpetuating state of the autoimmune response can be interrupted or even prevented, resulting in less severe joint damage (14). This could also include a drug-specific effect on antibody related disease processes.

A significant protective effect of DERAA carriership was not observed. However, in the cohort under study only a small minority of patients (n = 67; 17%) was DERAA-positive. Consequently, it cannot be ruled out that a protective effect would have been observed in group 1 (sequential monotherapy) if more DERAA-positive patients had been included (OR 0.4; 95% C.I. 0.1-1.2). Interestingly, for patients in group 3, who received initial combination therapy including an initial high dose of prednisone, there was no statistically significant difference in radiographic progression between patients with and without autoantibodies. For patients in group 4, who received initial combination therapy with infliximab, the difference between RF-positive and RF-negative patients and between ACPA-positive and ACPA-negative patients was statistically significant. Partially, this discrepancy may be explained by the fact that radiographic progression is lower in RF-negative and ACPA-negative patients in group 4 compared to similar patients in group 3. Indeed, the probability plots depicting the radiographic progression scores for patients in groups 3 and 4 show very similar results, with curves for patients with and those without antibodies almost overlapping (Figure 1B and 1C), suggesting that combination therapy with MTX is the crucial factor to overrule the association between radiographic progression and antibodies. As already pointed out by Landewe and colleagues (15), probability plots reveal important additional information about radiographic progression in patients, but cannot replace statistical testing. After correction for other baseline characteristics interfering with antibody status or with radiographic progression, RF and ACPA are only predictive for progressive disease with sequential monotherapy underlining that early combination therapy with MTX is warranted for RF-positive and ACPA-positive patients. Given the intensive treatment protocol in the BeSt study, resulting in treatment adjustments every 3 months to achieve DAS ≤ 2.4, it is difficult to evaluate drug-specific effects in the current context.

Based on the current data, we can conclude that for RA patients with an HLA-genotype or antibody profile traditionally associated with more severe joint damage progression, early combination therapy with MTX appears to overrule such an association and offers the best chance for prevention of joint damage progression.

REFERENCES

1. Berglin E, Johansson T, Sundin U, Jidell E, Wadell G, Hallmans G et al. Radiological outcome in rheumatoid arthritis is predicted by presence of antibodies against cyclic citrullinated peptide before and at disease onset, and by IgA-RF at disease onset. Ann Rheum Dis 2006; 65(4):453-458.
2. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987; 30(11):1205-1213.
3. Vos K, van der Horst-Bruinsma IE, Hazes JM, Breedveld FC, le Cessie S, Schreuder GM et al. Evidence for a protective role of the human leukocyte antigen class II region in early rheumatoid arthritis. Rheumatology (Oxford) 2001; 40(2):133-139.
4. van der Helm-van Mil AH, Huizinga TW, Schreuder GM, Breedveld FC, de Vries RR, Toes RE. An independent role of protective HLA class II alleles in rheumatoid arthritis severity and susceptibility. Arthritis Rheum 2005; 52(9):2637-2644.
5. Smolen JS. Autoantibodies in rheumatoid arthritis. In: van Venrooij WJ, Maini RN, editors. Manual of biological markers of disease. Dordrecht (The Netherlands): Kluwer Academic Publishers, 1996: 1-18.
6. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000; 43(1):155-163.
7. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 2004; 50(2):380-386.
8. Huizinga TW, Amos CI, van der Helm-van Mil AH, Chen W, van Gaalen FA, Jawaheer D et al. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. Arthritis Rheum 2005; 52(11):3433-3438.
9. van Gaalen FA, van Aken J, Huizinga TW, Schreuder GM, Breedveld FC, Zanelli E et al. Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis. Arthritis Rheum 2004; 50(7):2113-2121.
10. van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, de Jong BA, Breedveld FC, Verweij CL et al. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. Arthritis Rheum 2004; 50(3):709-715.
11. Lard LR, Boers M, Verhoeven A, Vos K, Visser H, Hazes JM et al. Early and aggressive treatment of rheumatoid arthritis patients affects the association of HLA class II antigens with progression of joint damage. Arthritis Rheum 2001; 46(4):899-905.
12. Goekoop-Ruiterman Y.P.M., De Vries-Bouwstra J.K., Van Zeben D, Kerstens P.J.S.M., Hazes J.M.W, Zwinderman A.H. et al. Treatment strategies in early rheumatoid arthritis: clinical and radiological outcomes after 2 year follow-up of the BeSt study. Arthritis Rheum 2004; 50(12):4096.

13. Ronnelid J, Wick MC, Lampa J, Lindblad S, Nordmark B, Klareskog L et al. Longitudinal analysis of citrullinated protein/peptide antibodies (anti-CP) during 5 year follow up in early rheumatoid arthritis: anti-CP status predicts worse disease activity and greater radiological progression. Ann Rheum Dis 2005; 64(12):1744-1749.
14. van Gaalen F, Ioan-Facsinay A, Huizinga TW, Toes RE. The devil in the details: the emerging role of anticitrulline autoimmunity in rheumatoid arthritis. J Immunol 2005; 175(9):5575-5580.
15. Landewe R, van der Heijde D. Radiographic progression depicted by probability plots: presenting data with optimal use of individual values. Arthritis Rheum 2004; 50(3):699-706.

Chapter 10

Summary and discussion

In this thesis, the effectiveness of different treatment strategies in early rheumatoid arthritis is evaluated. Tailor-made treatment of the individual patient with early rheumatoid arthritis is possible if both disease course and treatment response can be accurately predicted at the moment of presentation. Therefore, several clinical, serologic, and genetic markers are studied for their association with treatment response and/ or a more severe disease course.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a complex autoimmune disease with a variable course and outcome. The main feature is arthritis of multiple joints eventually resulting in destruction of cartilage and bone. A general introduction on RA and a brief history of the therapeutic approach to RA are given in chapter 1. Nowadays, treatment with Disease Modifying Antirheumatic Drugs (DMARDs) is started directly after diagnosis, resulting in more effective suppression of disease activity and substantial reduction of joint damage.

Prediction of radiographic damage

Given the variable course and outcome of RA many studies have focused on markers that can predict a more severe disease course. As shown by Table 2, chapter 1, baseline radiographic score, presence of autoantibodies, and serologic evidence of disease activity are consistently found to be predictive of more severe progression of joint damage. In chapter 2, it is studied whether a prediction model, based on all currently known prognostic factors for joint damage progression, can be useful to make treatment decisions for individual patients presenting with RA. For this goal, a cohort of patients that presented with very early RA between 1993 and 1999 was selected from the Leiden Early Arthritis Clinic. According to current standards, the selected cohort was undertreated. During the first year of follow-up only 85% of patients were treated with DMARDs with antimalarial drugs being prescribed most often, resulting in 72% of patients with progression of joint damage. The developed model had a positive predictive value (PPV) of 75% indicating that, retrospectively, in 75% of patients the presence or absence of radiographic progression could be predicted correctly. However, when taking into account that at least 80% certainty is needed in a prospective situation, the model was useful for balanced treatment decisions in only 46% of patients. Patients without progression of joint damage could not be reliably identified with this model.

The power of the predictive model was comparable to models developed in other cohorts (Table 2, chapter 1; Table 6, chapter 2). Analysis of hypothetical prediction models with better prognostic power showed that differentiated treatment decisions would have been possible in 80% of patients if better prognostic markers were available, urging the search for new markers.

Biologic agents in early RA

The introduction of the so-called *biologics* has led to a revolutionary change in the treatment of RA. The term refers to a group of therapeutic agents that specifically target a particular cell or

cytokine important in the inflammatory process in RA. Especially with the biologics inhibiting TNF-α, —infliximab, etanercept and adalimumab— , prevention or even improvement of joint damage seems to be possible. In chapter 3, the role of TNF-α inhibitors in the treatment of early RA is evaluated. Several randomized, placebo-controlled trials have compared the efficacy of a TNF-α inhibitor, methotrexate, and the combination of a TNF-α inhibitor and methotrexate in early stage RA. These trials all showed the superiority of the combination of a TNF-α inhibitor and methotrexate, with a greater proportion of patients achieving clinical remission and less progression of joint damage, as compared to monotherapy. However, the efficacy of methotrexate alone was also underlined, with remission achieved in 15% - 25% of patients with poor prognosis RA. As the TNF-α inhibitors are far more expensive than traditional DMARDs, and long-term safety data for the subgroup of early RA patients are not yet available, additional observations have to clarify the balance in terms of cost and benefit for the individual patient with early RA.

Remission-induction therapy

There are several indications that immediate treatment with a TNF-α inhibitor might be even more beneficial than reserving such treatment for patients who have failed on traditional DMARDs. Quinn and colleagues showed that initial treatment with infliximab in early, poor prognosis RA results in sustained improvement of outcome, even after completely stopping treatment with infliximab.

These observations raise the question which treatment strategy should be employed in patients presenting with RA.

The BeSt study

In chapter 4 and 5, the primary outcomes of the BeSt (Dutch acronym for treatment strategies: Behandelstrategieën) study are described. Rather than comparing (combinations of) drugs, this randomized, single-blinded, clinical trial compared four treatment strategies in 508 patients with newly diagnosed, active RA:

Group 1, sequential monotherapy (n = 126), where patients started with one DMARD (methotrexate), which was replaced by subsequent other monotherapies in case of insufficient response;

Group 2, step-up combination therapy (n = 121), where patients started with one DMARD (methotrexate), but, if necessary, more DMARDs were added step by step;

Group 3, initial combination therapy (n = 133), where patients started directly with a combination of methotrexate, sulphasalazine, and an initial high-dose prednisone (60 mg, tapered to 7.5 mg during the first 7 weeks), followed by other combinations in case of insufficient response;

Group 4, initial combination therapy including a TNF-α inhibitor (n = 128), where patients started with a combination of the TNF-α inhibitor infliximab and methotrexate.

Patients in groups 1, 2, and 3 could also be treated with a combination of methotrexate and infliximab, but only after failure on at least three 'conventional' DMARDs.

The common goal in all strategies was to reduce disease activity rapidly and persistently by tight monitoring and immediate adjustment of therapy in case of an insufficient response (DAS > 2.4). Every 3 months, the DAS was calculated by a research nurse blinded for the allocated treatment group. In case of insufficient response, the rheumatologist adjusted the medication as dictated by the pharmacoprotocol of the particular treatment group. If the clinical response was consistently adequate (DAS ≤ 2·4 for at least 6 months), medication was tapered until only one drug remained in a maintenance dose.

Outcomes of the BeSt study

Functional ability improved significantly more quickly in the groups starting with combination therapy including either prednisone or infliximab. After 3 months, the HAQ score had improved from 1.4 to 0.6 in groups 3 and 4, and from 1.4 to 1.0 in groups 1 and 2. During the remaining period of the first year, the HAQ further improved in all groups, remaining significantly lower in groups 3 and 4 than in group 1. During the second year of follow-up, no significant further improvement was seen in functional ability, with mean HAQ at 2 years of follow-up of 0.7, 0.6, 0.5, and 0.5 for groups 1 – 4, respectively ($P = 0.257$).

Reduction of disease activity was seen earlier in the groups receiving initial combination therapy including either prednisone or infliximab. After one year, clinical response was comparable in all groups. After 2 years of follow-up, 42% of all patients had achieved clinical remission (DAS < 1.6).

In groups 1 and 2, to achieve low disease activity, treatment had to be adjusted more often than in groups 3 and 4. After 2 years, 33% of patients with sequential monotherapy and 31% of patients with step-up combination therapy were still treated according to the first step of the pharmacoprotocol: methotrexate monotherapy. Of all patients with initial combination therapy including prednisone, 58% was still in the first treatment step, and of the patients with initial combination therapy including infliximab, 72% was still in the first treatment step.

After 1 year as well as after 2 years, progression of radiographic damage was significantly less in groups 3 and 4 compared with groups 1 and 2. After 2 years, the progression in total SHS was 2.0, 2.0, 1.0, and 1.0, respectively (group 1 vs. 2, $P = 0.850$; group 1 vs. 3, $P = 0.043$; group 1 vs. 4, $P = 0.014$; group 2 vs. 3 , $P = 0.006$; group 2 vs. group 4, $P = 0.004$; group 3 vs. 4, $P = 0.798$). Over 2 years, significantly more patients in group 1 (40%) and 2 (34%) had progressive disease defined as SHS progression with more than the smallest detectable change (SDC; 4.64 points), as compared to patients in groups 3 (20%) and 4 (18%).

There were no significant differences in the number of adverse events or serious adverse events between the four treatment groups during the first 2 years of treatment.

In conclusion, patients treated with initial combination therapy, including either a tapered high-dose of prednisone or infliximab, achieve low disease activity earlier than patients treated with initial methotrexate monotherapy, have earlier improvement of

functional ability, and less progression of joint damage, and do not experience more adverse events. Over 2 years of follow-up, no statistically significant differences in the clinical and radiographic outcomes were found that indicate that one initial combination regimen would be superior over the other.

The importance of combination therapy

The results of the BeSt study show a clear association between early clinical remission and prevention of joint damage progression. Previous studies have shown that treatment with infliximab results in suppression of joint damage regardless of clinical efficacy. To study whether suppression of joint damage could be due to a drug effect of infliximab or prednisone, we compared patients treated with initial combination therapy (groups 3 and 4) with patients treated with initial monotherapy (groups 1 and 2), dividing each group into 2 subgroups: patients who achieved early and continuous clinical remission and patients who continuously failed to achieve a sufficient clinical response. The results of this analysis are presented in chapter 6.

It was obvious that continuous remission occurred more often in the initial combination therapy groups and was associated with less joint damage progression. Patients who achieved continuous remission on initial combination therapy showed significantly less often joint damage progression than patients who achieved continuous remission on initial monotherapy. Partly this may be due to the fact that patients treated with initial combination therapy achieve remission earlier than patients treated with initial methotrexate monotherapy, but a specific drug effect cannot be ruled out. Interestingly, patients who failed on initial combination therapy still appeared to have some benefit of the combination. Compared to patients who failed on initial monotherapy, failures on combination therapy showed better functional ability.

Prognostic markers: cytokines

Many proinflammatory and antiinflammatory cytokines have been found at the major site of RA, the synovium. Physiologic inflammatory responses are naturally transient. In contrast, the inflammatory response in RA synovium has a chronic character and an imbalanced cytokine profile with a relative excess of TNF-α and interleukin 1 (IL1). This observation contributed to the development of therapeutic TNF-α inhibitors and recombinant interleukin 1 receptor antagonist. The idea that part of the unrevealed genetic background of RA may be found in genetically determined differences in cytokine production was directed by twin studies that showed a considerable heritability (50% - 80%) of cytokine production.

Interleukin 1 β and Interleukin 1 receptor antagonist

The proinflammatory cytokine interleukin 1 β (IL1β) plays an important role in perpetuating the inflammatory and destructive processes in the rheumatoid joint.

Interleukin 1 receptor antagonist (IL1Ra) is the most important physiological regulator of IL1β activity. To study the association between the capacity to produce IL1β and IL1Ra and susceptibility to and severity of RA, we determined ex vivo lipopolysaccharide (LPS) induced IL1β and IL1Ra production in 76 early RA patients from the BeSt study and in 63 healthy controls. In chapter 7, we show that high IL1Ra production and low IL1β production are associated with diagnosis and severity of RA. The differences in IL1β production could not be explained by different distribution of the Single Nucleotide Polymorphism (SNP) C-511T that was previously shown to be associated with IL1β production.

The increased IL1Ra production and decreased IL1β production in RA patients seem counterintuitive. Despite the clear reliability of the assay used, we do not know how the ex vivo production levels relate to actual circulating levels in vivo. Locally, a considerable excess of IL1Ra is needed to counterbalance the effects of IL1β receptor binding. Thus, the excess of IL1Ra might not be enough.

In addition, high IL1Ra production was strongly associated with radiographic progression in these aggressively treated RA patients, also after correction for known covariates. Overall, the current results point at a key role for IL1β and IL1Ra in the pathogenesis of RA, but further research is needed for exact interpretation of the pathogenetic background of our observations.

Prognostic markers: genetics and autoantibodies

The HLA DRB1 alleles encoding for the shared epitope (SE) are the strongest known genetic risk factor for development of RA. In addition, a clear association has been shown between presence of SE and severity of radiographic progression in RA. Presence of autoantibodies, notably rheumatoid factor (RF), and anti-cyclic citrullinated peptide antibodies (ACPA), is consistently reported to be one of the strongest predictors of progression of joint damage in RA.

Interestingly, Lard and colleagues showed that by treating RA early and aggressively, the association between HLA DRB1 and joint damage disappeared while the association between RF and joint damage was still observed.

HLA DRB1, RF and ACPA in the BeSt cohort

To further explore the influence of different treatment strategies on the association between HLA DRB1, RF, ACPA, and progression of joint damage in early RA, data were compared between the patients in the four treatment groups of the BeSt study. In chapter 9, it is shown that carriership of SE did not increase the risk for radiographic progression in any of the treatment groups. Univariate analysis of the association between presence of RF and ACPA with radiographic progression showed significantly higher progression scores for patients with autoantibodies in groups 1, 2, and 4. After correction for other variables interfering with this association, the risk for progressive disease was only increased for patients with RF or ACPA in group 1, sequential monotherapy. Thus,

although autoantibodies are the strongest known prognostic factor for more severe radiographic damage, early combination therapy including methotrexate seems to overrule this association. These data suggest that, with early and aggressive intervention, a self-perpetuating state of the autoimmune response can be interrupted or even prevented, resulting in less severe joint damage.

Pharmacogenetics to predict methotrexate effectiveness

Methotrexate is the most commonly used DMARD for treatment of RA given its proven efficacy, its favorable price, and the wide experience with its use, also as part of combination regimens. Nevertheless, clinical efficacy of methotrexate is variable and toxicity results in discontinuation of treatment in up to 30% of patients. There are no useful and reliable clinical markers that can predict response to methotrexate.

Pharmacogenetics is the study of genetic polymorphisms in drug-metabolizing enzymes and the translation of inherited differences into differences in effectiveness of drugs. Recent developments in the field of genetic research enabled rapid and accurate detection of large numbers of genetic polymorphisms, and thus enable detection of single nucleotide polymorphisms (SNP) that are responsible for variability in drug responses between individual patients.

Methotrexate, a folate analog, has shown to influence the intracellular folate metabolism. SNPs in genes encoding folate pathway enzymes, including (among others) dihydrofolate reductase (DHFR), methylenetetrahydrofolate reductase (MTHFR), and reduced folate carrier (RFC), have been shown to influence methotrexate efficacy and toxicity but results are variable and sometimes conflicting.

SNPs in MTHFR, DHFR and RFC genes in the BeSt cohort

To determine the association of methotrexate efficacy and toxicity with SNPs in MTHFR, DHRF, and RFC genes, all patients in the BeSt cohort that started with methotrexate monotherapy (groups 1 and 2) were selected and the following SNPs were analyzed: MTHFR 677C>T, MTHFR 1298A>C, DHFR –473G>A, DHFR 35289G>A, and RFC 80G>A. In chapter 8, we show that good clinical improvement (ΔDAS > 1.2) was more likely for patients typed as MTHFR 1298AA as compared to C-allele carriers (OR = 2.3, 95% CI 1.18-4.41). A haplotype consisting of MTHFR 1298AA and MTHFR 677CC showed even stronger association with MTX efficacy: 76.9% of patients with the haplotype showed good clinical improvement as compared to 64.2% of all other patients. In contrast, in MTHFR 1298 C-allele carriers side effects were more common (OR = 2.5, 95% CI 1.32-4.72). For the other SNPs no association with toxicity or efficacy was observed.

Our results are partly in contrast to previously published associations. These differences can probably be explained by differences in patient selection, pharmacoprotocol and prescription of folic acid next to methotrexate. For treatment with methotrexate – which affects folate metabolism via several pathways — in a complex disease like RA, it is in fact unlikely that a single SNP will be sufficient to adequately predict treatment efficacy. More

likely, a composite of different risk loci will have to be determined to be able to identify patients with high risk for MTX inefficacy or toxicity. The current data show that MTHFR genotypes are suitable candidates as part of such a risk profile and thus in the future may help determine which patients will benefit most from MTX treatment.

Which treatment strategy is the BeSt?

The evaluation of different treatment strategies in early RA as described in chapters 3 - 6 of this thesis clearly shows that initial combination therapy including methotrexate and either a tapered high-dose of prednisone or a TNF-α inhibitor is superior to initial monotherapy. Nevertheless, all strategies as applied in the BeSt study resulted in remarkable improvement of the participating patients. Although the patients were selected for active, more severe disease at baseline (mean DAS 4.4, 72% evidence of erosive disease), clinical remission was achieved in 42% of patients and median progression scores were low in all 4 groups. These results may partly be attributed to the tight control as achieved by intensive monitoring and immediate adjustment of medication (1).

The beneficial effect of tight monitoring of disease activity has been confirmed by other studies (2,3). In the TICORA study performed by Grigor and colleagues, even 65% of patients achieved clinical remission after 18 months which can serve as an argument for monthly monitoring as performed in this study. On the other hand, median progression scores were higher (4.5 after 18 months) as compared with the BeSt study, also in the group that was tightly monitored, indicating that apart from frequent monitoring of disease activity, also the treatment strategy of choice is crucial for outcome.

After 2 years of follow-up in the BeSt study, the absolute difference in median radiographic progression between all 4 groups is low (2 for groups 1 and 2; 1 for groups 3 and 4), which could raise the question whether this difference is clinically relevant. Several studies have indicated that aggressive intervention early in the disease course might be more effective in suppressing progression of joint damage than aggressive treatment after failure on previous treatment (4,5). Long-term follow-up of patients in the BeSt study will show if this is also true for the strategies studied in this cohort. The fact that patients in the BeSt study, who initially started with a combination of drugs, show less joint damage progression than patients who started with initial monotherapy, despite comparable DAS levels after one year, underlines the differential effectiveness of early aggressive intervention. Notably, patients who achieved a DAS ≤ 2.4 on initial combination therapy all tapered treatment to monotherapy (either methotrexate or sulphasalazine) after 9 months and still showed superior suppression of joint damage progression after two years compared with patients who achieved a DAS ≤ 2.4 on initial methotrexate monotherapy, indicating that early induction of low disease activity has a prolonged effect. In group 3, the initial combination regimen was tapered successfully to monotherapy in 1/3 of patients, in group 4, infliximab could be stopped permanently in more than 50% of patients without a flare of disease activity or increase in progression of joint damage (6-8).

In contrast, of all patients who started with initial methotrexate monotherapy in group 1, 27% had started with infliximab during 2 years of follow-up because of failure on previous treatment steps, together with 7% in group 2 and 13% in group 3. Long-term follow-up of these patients will have to point out whether treatment with infliximab started later in the disease course shows comparable efficacy with initial treatment with infliximab, and can be discontinued as often.

Thus far, none of the presented analyses demonstrates clear superiority of one combination regimen over the other. Obviously, prescribing a combination of prednisone, methotrexate and sulphasalazine is initially cheaper than prescribing infliximab. However, since over 50% of patients who started with infliximab can successfully stop infliximab treatment, and many patients in the other groups start treatment with infliximab in the course of time, in the long term the costs of initial infliximab treatment might be a worthwhile investment (9,10). Regarding the combination including the initial high dose of prednisone, several considerations are of interest. Thus far, we do not know which element of the prescribed combination is crucial for the observed effectiveness. Several studies showed a beneficial effect of adding low-dose prednisone to traditional DMARDs (11,12), indicating that an initial high dose may not be required. Interestingly, a molecular study on the precise mechanism of action of methotrexate and sulphasalazine gave evidence for probable counteraction of these two DMARDs (13). Currently, studies are being undertaken to explore this issue. In the short term, side effects of prednisone appear to be acceptable, but again, in the long term this needs to be re-evaluated.

How to treat the individual RA patient?

Chapters 2, 7 - 9, have focused on prognostic markers in early RA and the possibility to make balanced treatment decisions for the individual patient presenting with RA. Reviewing the data from the BeSt study, it has been argued that, when starting with a combination of drugs in all patients that present with RA, a considerable proportion would have been 'overtreated' (14). Indeed, after 2 years of follow-up approximately one-third of patients that started with methotrexate alone still shows good clinical response. Several of the currently described observations argue against starting with methotrexate alone in (a subgroup of) RA patients.

First, characteristics which have been associated with a more aggressive disease course seem to be overruled when starting with a combination of drugs (chapter 9). This observation is confirmed by data from the Aspire study that compared the efficacy of methotrexate with the combination of methotrexate and infliximab in early RA (15). Categorizing patients by baseline C-reactive protein (CRP) level and erythrocyte sedimentation rate (ESR), the investigators showed that patients on methotrexate with high CRP and/or high ESR had more severe radiographic progression than patients with normal CRP and ESR. As a whole, the infliximab group experienced less radiographic progression than the methotrexate-only group, in spite of the level of baseline inflammation markers. Thus, based on these data patients with characteristics for more severe disease should all start with combination therapy.

Alternatively, we might want to identify those patients with less severe disease, ie, those who would not need combination therapy. In chapter 2 it is shown that, given the severe nature of RA, the risk of undertreatment is always higher than the risk of overtreatment. This chapter also showed that starting with patients diagnosed with definite RA, no reliable prediction can be made identifying those patients that will not have joint damage progression. Current models developed in other cohorts do not show better discriminating powers (See Table 2, chapter 1).

Finally, two novel 'prognostic factors' (IL1Ra, MTHFR/RFC/ DHRF genes) were studied but for both further research is needed before they can prove their efficacy in clinical practice.

Towards tailor-made treatment of early RA

In conclusion, starting from the current situation, a case can be made for initial combination therapy in all patients presenting with RA. In addition, to improve outcome in the best possible way, rheumatologists and patients should define high treatment goals and evaluate success or failure regularly. In the mean time, the search for better tools to predict disease outcome and treatment response is warranted.

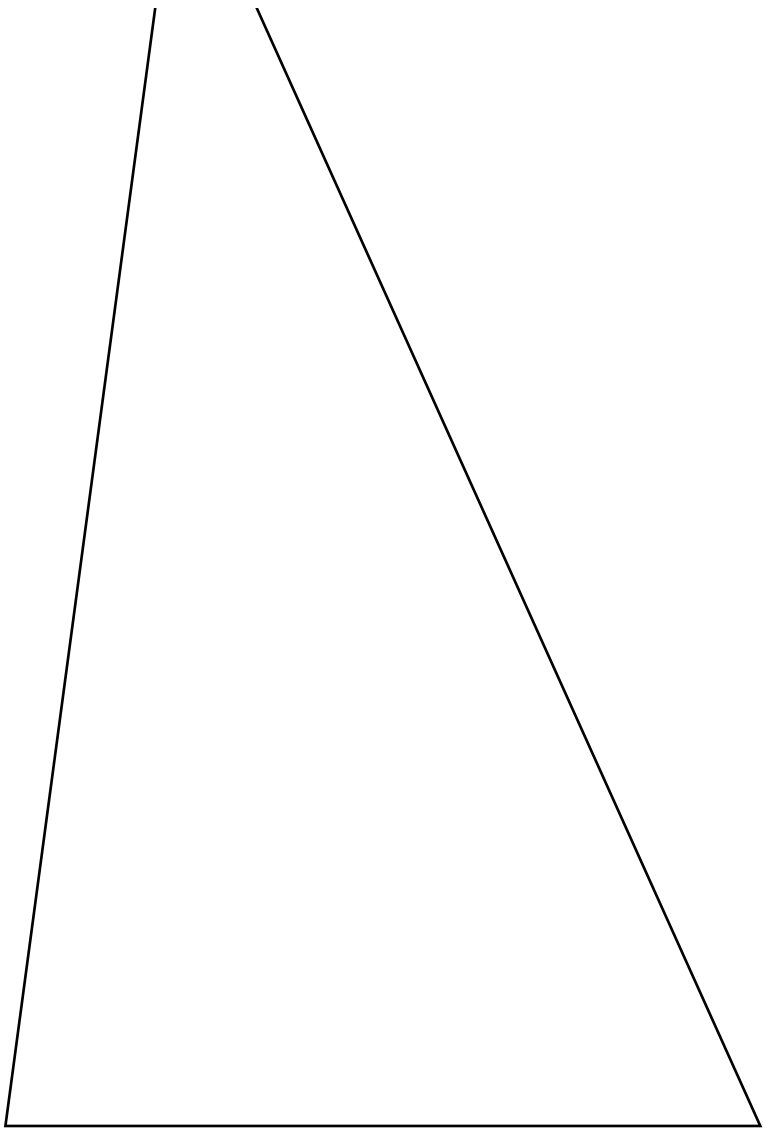
REFERENCES

1. Goekoop-Ruiterman YPM, de Vries-Bouwstra JK, Allaart CF, Nielen MMJ, Kerstens PJSM, van Schaardenburg D et al. Efficacy of DAS-driven therapy versus routine care in patients with recent onset active rheumatoid arthritis [abstract]. Arthritis Rheum 2006; 54(9 (Suppl.)):S372-S373.
2. Fransen J, Moens HB, Speyer I, van Riel PL. Effectiveness of systematic monitoring of rheumatoid arthritis disease activity in daily practice: a multicentre, cluster randomised controlled trial. Ann Rheum Dis 2005; 64(9):1294-1298.
3. Grigor C, Capell H, Stirling A, McMahon AD, Lock P, Vallance R et al. Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomised controlled trial. Lancet 2004; 364(9430):263-269.
4. Baumgartner SW, Fleischmann RM, Moreland LW, Schiff MH, Markenson J, Whitmore JB. Etanercept (Enbrel) in patients with rheumatoid arthritis with recent onset versus established disease: improvement in disability. J Rheumatol 2004; 31(8):1532-1537.
5. Quinn MA, Conaghan PG, O'Connor PJ, Karim Z, Greenstein A, Brown A et al. Very early treatment with infliximab in addition to methotrexate in early, poor-prognosis rheumatoid arthritis reduces magnetic resonance imaging evidence of synovitis and damage, with sustained benefit after infliximab withdrawal: results from a twelve-month randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2005; 52(1):27-35.
6. van de Bijl AE, van der Kooij SM, Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Breedveld FC, Allaart CF et al. Persistent good clinical response after tapering and discontinuation of initial infliximab therapy in patients with early rheumatoid arthritis: 3-year results from the BeSt-trial [abstract]. Ann.Rheum.Dis. 65[Supplement II], 109. 1-7-2006.
7. van der Kooij SM, van de Bijl AE, Allaart CF, Goekoop-Ruiterman YPM, de Vries-Bouwstra JK, Gerards AH et al. Remission induction in early Rheumatoid Arthritis (RA) with initial infliximab (IFX) and methotrexate (MTX) therapy: The disease course after IFX discontinuation in the BeSt trial [abstract]. Arthritis Rheum 2006;(9 (Suppl.)):S302-S303.
8. van der Kooij SM, Allaart CF, van de Bijl AE, Goekoop-Ruiterman YPM, de Vries-Bouwstra JK, van Groenendael JHLM et al. Induction of remission in patients with early Rheumatoid Arthritis (RA) initially treated with either prednisone or infliximab combination therapy [abstract]. Arthritis Rheum 2006; 54(9 (Suppl.)):S542.
9. Puolakka K, Kautiainen H, Mottonen T, Hannonen P, Korpela M, Julkunen H et al. Impact of initial aggressive drug treatment with a combination of disease-modifying antirheumatic drugs on the development of work disability in early rheumatoid arthritis: a five-year randomized followup trial. Arthritis Rheum 2004; 50(1):55-62.
10. van den Hout WB, Goekoop-Ruiterman YPM, Allaart CF, de Vries-Bouwstra JK, van Zeben D, Hazes JMW et al. Additional costs of initial therapy including infliximab (IFX) in patients with early rheumatoid arthritis (RA) may be compensated by the value of sustained working hours: 2 year results of the BeSt study [abstract]. Arthritis Rheum 2006; 54(9 (Suppl.)):S228-S229.
11. Svensson B, Boonen A, Albertsson K, van der Heijde D.M., Keller C, Hafstrom I. Low-dose prednisolone in addition to the initial disease-modifying antirheumatic drug in patients with early active rheumatoid arthritis reduces joint destruction and increases the remission rate: a two-year randomized trial. Arthritis Rheum 2005; 52(11):3360-3370.

12. Wassenberg S, Rau R, Steinfeld P, Zeidler H. Very low-dose prednisolone in early rheumatoid arthritis retards radiographic progression over two years: a multicenter, double-blind, placebo-controlled trial. Arthritis Rheum 2005; 52(11):3371-3380.
13. Jansen G, van der Heijden J, Oerlemans R, Lems WF, Ifergan I, Scheper RJ et al. Sulfasalazine is a potent inhibitor of the reduced folate carrier: implications for combination therapies with methotrexate in rheumatoid arthritis. Arthritis Rheum 2004; 50(7):2130-2139.
14. Zeidler H, Hulsemann JL. How should best strategy and tight control be translated into clinical practice? Comment on the article by Goekoop-Ruiterman et al. Arthritis Rheum 2006; 54(7):2338-2339.
15. Smolen JS, van der Heijde DM, St Clair EW, Emery P, Bathon JM, Keystone E et al. Predictors of joint damage in patients with early rheumatoid arthritis treated with high-dose methotrexate with or without concomitant infliximab: results from the ASPIRE trial. Arthritis Rheum 2006; 54(3):702-710.

Chapter 11

Samenvatting en bespreking



DE WEG NAAR BEHANDELING OP MAAT VOOR PATIENTEN MET BEGINNENDE REUMATOÏDE ATRITIS

In dit proefschrift wordt de effectiviteit van verschillende behandelstrategieën voor patiënten met recent gediagnostiseerde reumatoïde artritis geëvalueerd. In de optimale situatie zou elke patiënt met reumatoïde artritis direct die behandeling moeten krijgen die voor hem/ haar het meest effectief is: behandeling op maat. Behandeling op maat is alleen dan mogelijk als zowel het beloop van de ziekte als de reactie op verschillende geneesmiddelen betrouwbaar kunnen worden voorspeld. Daarom worden in dit proefschrift verschillende klinische, serologische en genetische factoren besproken die invloed hebben op het beloop van reumatoïde artritis en op de reactie op geneesmiddelen.

Reumatoïde artritis

Reumatoïde artritis is een chronische ontstekingsziekte waarbij met name de kleine gewrichten van de handen en voeten zijn aangedaan. Ontstekingsreacties kunnen echter ook optreden in andere gewrichten en in andere organen zoals het hart, de kleine bloedvaten, de nieren en de longen.

Tot op heden is het nog niet bekend waardoor reumatoïde artritis (RA) wordt veroorzaakt. Wel is duidelijk dat RA een auto-immuunziekte is. Bij auto-immuunziekten begint het afweersysteem van het lichaam een ontstekingsreactie tegen lichaamseigen cellen en weefsels. De belangrijkste ontstekingsreactie bij RA speelt zich af in het gewrichtskapsel. Dit leidt tot zwelling, pijn en verminderde beweeglijkheid van het gewricht. Als het gewrichtskapsel gedurende langere tijd ontstoken is, kan dit uiteindelijk het aangrenzende kraakbeen en bot beschadigen. Deze schade aan kraakbeen en bot is te zien op röntgenfoto's van het gewricht als versmalling van de gewrichtsspleet en als oppervlakkige onderbrekingen in het bot (erosies).

Naast pijnlijke en stijve gewrichten, hebben patiënten met RA vaak last van algemene ziekteverschijnselen, zoals moeheid, temperatuursverhoging en gewichtsverlies. Hoe de ziekte verloopt, verschilt van patiënt tot patiënt: bij sommige patiënten zijn slechts enkele gewrichten ontstoken en treedt niet of nauwelijks schade van kraakbeen en bot op, terwijl bij de meeste patiënten voortdurend meerdere gewrichten ontstoken zijn en steeds meer schade ontstaat. Voor de individuele patiënt varieert het ziektebeeld in de tijd, met perioden van actieve ziekte met veel gewrichtsontstekingen en perioden waarin de ziekte rustiger lijkt.

In Nederland lijden ongeveer 160.000 mensen aan RA. De ziekte komt vaker voor bij vrouwen dan bij mannen en begint meestal tussen het 40e en het 60e jaar. Doordat de bevolking in Nederland gemiddeld steeds ouder wordt, is de verwachting dat het aantal mensen dat aan RA lijdt in de komende jaren zal toenemen.

Risicofactoren

Onderzoek naar de mogelijke oorzaken van RA heeft laten zien dat erfelijke factoren een rol spelen bij het ontstaan van RA. Dit blijkt uit het feit dat RA vaker voorkomt

bij eerstegraads familieleden van patiënten met RA dan bij de gemiddelde bevolking. De bekendste genetische risicofactor ligt in de HLA-genen (Human Leukocyte Antigen). HLA-genen spelen een rol in het afweersysteem en bepalen mede of een afweercel een andere cel ziet als 'vijandelijk' of als lichaamseigen. Een bepaalde combinatie van HLA-genen, ook wel genaamd 'shared epitope', komt veel vaker voor bij RA patiënten dan bij anderen. Bij RA patiënten die deze combinatie van HLA-genen hebben, ontstaat bovendien vaak ernstiger schade aan de gewrichten dan bij RA patiënten zonder deze combinatie van HLA-genen. Behalve de HLA-genen dragen ook andere genen bij aan het ontstaan van RA. Welke dat zijn, is op dit moment onderwerp van verschillende studies.

Een kenmerk van RA is dat bij veel patiënten autoantistoffen in het bloed te vinden zijn. Bij gezonde personen maken de afweercellen antistoffen tegen schadelijke 'indringers' zoals bacteriën en virussen. De antistoffen binden aan de ziekteverwekkers zodat de afweercellen weten welke cellen opgeruimd moeten worden. Autoantistoffen binden zich aan lichaamseigen cellen of weefsels waardoor het afweersysteem deze eigen cellen en weefsels aanvalt. De eerste autoantistof die bij RA-patiënten werd gevonden is reumafactor. Meer recent werd een andere autoantistof gevonden: de anti-CCP antistof. RA patiënten met autoantistoffen hebben een grotere kans op ernstige gewrichtsschade dan RA patiënten zonder autoantistoffen.

Evaluatie van ziekteactiviteit

Om op een objectieve manier te meten hoe actief/ ernstig RA bij een individuele patiënt verloopt en het effect van de behandeling te beoordelen, zijn verschillende meetmethoden ontwikkeld. Om de mate van gewrichtsontsteking te beoordelen worden de afzonderlijke gewrichten onderzocht op pijn en zwelling. Samen geeft dit de gewrichtsscore. Een andere maat voor ontsteking is de bloedbezinkingssnelheid (BSE) of het CRP-gehalte in het bloed.

De DAS (Disease Activity Score) is een getal dat wordt berekend uit de gewrichtsscore, de BSE en de mening van de patiënt over zijn/ haar gezondheid. Hoe hoger de DAS, des te actiever de ontsteking. Een DAS lager dan of gelijk aan 2,4 komt overeen met 'lage ziekteactiviteit'; een DAS lager dan 1,6 betekent dat er geen meetbare ontstekingsactiviteit meer is, oftewel remissie van ziekte.

Als weerspiegeling van de ziekteactiviteit kan met behulp van gestandaardiseerde vragenlijsten worden beoordeeld in hoeverre een patient in zijn/ haar dagelijks functioneren wordt beperkt door de RA. Een veelgebruikte vragenlijst is de HAQ (Health Assessment Questionnaire). Hoe hoger de HAQ score, hoe meer beperkingen.

Een ernstig gevolg van RA is onherstelbare schade aan de gewrichten. Door herhaaldelijk röntgenfoto's te maken van handen en voeten en deze op een gestandaardiseerde manier met elkaar te vergelijken, wordt beoordeeld of de behandeling de progressie van schade voldoende tegen gaat. Een manier om de schade systematisch te beoordelen is de Sharp-van der Heijde methode. De score, dikwijls afgekort als SHS, loopt van 0 tot 448 punten.

Behandeling van patiënten met RA

De laatste jaren is de manier waarop RA behandeld wordt ingrijpend veranderd. Terwijl vroeger eerst behandeld werd met een hoge dosis pijnstillers om de ontstekingen te onderdrukken en de pijn te verminderen, wordt tegenwoordig direct gestart met zogenaamde Disease Modifying Antirheumatic Drugs (DMARDs). Dit zijn ontstekingsremmende geneesmiddelen die, juist als ze al vroeg in de ziekte worden gegeven, het verloop van de ziekte positief kunnen beinvloeden zodat minder schade aan de gewrichten optreedt. Bij gebruik van deze DMARDs duurt het enige weken tot maanden voordat het effect merkbaar is. Van tevoren is het niet te voorspellen welke patiënt goed zal reageren op welk middel of welke patiënt juist bijwerkingen zal krijgen van een bepaald middel. Ook kan na een aanvankelijk goed effect van een bepaald DMARD de ziekte op den duur toch weer opvlammen. Methotrexaat is wereldwijd op dit moment het meest voorgeschreven DMARD. Ook dit middel werkt slechts bij een deel van de patiënten, terwijl ongeveer 30% er uiteindelijk mee moet stoppen vanwege bijwerkingen.

Omdat juist vroeg en effectief onderdrukken van de ontstekingsreactie belangrijk is om schade aan de gewrichten zoveel mogelijk te voorkomen, worden ook combinaties van DMARDs voorgeschreven. Vergelijkende onderzoeken hebben laten zien dat het direct na de diagnose voorschrijven van combinaties van DMARDs resulteert in minder gewrichtsschade. Een Nederlandse studie, de COBRA studie, vergeleek behandeling met een combinatie van methotrexaat, sulfasalazine en een initieel hoge dosis prednison, die gedurende de eerste 7 weken werd afgebouwd, met behandeling met alleen sulfasalazine. Alle patiënten in de studie waren nog niet eerder behandeld voor RA. Terwijl de combinatie van middelen in de eerste maanden werd afgebouwd tot alleen sulfasalazine, hadden de patiënten die daarmee waren gestart ook na 5 jaar minder gewrichtsschade dan de anderen.

Nieuwe geneesmiddelen voor RA: 'biologics'

In de afgelopen jaren is een aantal nieuwe geneesmiddelen ontwikkeld voor de behandeling van RA. Gemeenschappelijk kenmerk van deze geneesmiddelen is dat ze specifiek gericht zijn tegen een bepaalde cel of stof die van belang is bij de ontstekingsreactie die optreedt bij RA; vandaar de naam 'biologics'. De geneesmiddelen die de ontstekingsstimulerende stof TNF-α blokkeren, te weten infliximab, etanercept en adalimumab, vormen een belangrijke groep binnen de 'biologics'. In tegenstelling tot de oudere DMARDs hebben deze geneesmiddelen een direct en sterker ontstekingsonderdrukkend effect waardoor gewrichtsschade voorkomen kan worden. Bovendien lijkt bij behandeling met TNF-α blokkers in sommige gevallen al ontstane, geringe, schade zich te kunnen herstellen.

In hoofdstuk 3 wordt de plaats van de TNF-α blokkers in de behandeling van recent gediagnosticeerde, ernstige RA besproken. Verschillende onderzoeken hebben laten zien dat een combinatie van een TNF-α blokker en methotrexaat effectiever is dan een TNF-α blokker of methotrexaat alleen in het onderdrukken van ontsteking en het voorkomen van schade. Toch lukte het bij 15 - 25% van deze groep patiënten met ernstige RA om met alleen methotrexaat de ziekte volledig te onderdrukken. Omdat van de TNF-α blokkers

nog weinig bekend is over bijwerkingen op lange termijn, en omdat deze middelen veel duurder zijn dan de traditionele DMARDs, is het dus nog de vraag of TNF-α blokkers meteen moeten worden voorgeschreven aan alle patiënten met RA, of dat ze 'bewaard' kunnen worden voor patiënten die niet goed reageren op alleen methotrexaat en de andere traditionele DMARDs. Ook is onduidelijk of de TNF-α blokkers effectiever zijn dan een combinatie van traditionele DMARDs.

BeSt studie

In hoofdstuk 4 en 5 worden de resultaten van de BeSt studie besproken. De afkorting BeSt staat voor Behandelstrategieëen. In dit gerandomiseerde onderzoek werden 4 behandelstrategieën vergeleken bij 508 patiënten met beginnende en actieve RA:

Groep 1, *sequentiële monotherapie (n = 126)*, waar patiënten startten met 1 DMARD (methotrexaat) dat bij onvoldoende effect werd vervangen door steeds 1 ander DMARD;

Groep 2, stap-op combinatie therapie (n = 121), waar patiënten startten met 1 DMARD (methotrexaat), waar, in geval van onvoldoende effect, andere DMARDs één voor één aan werden toegevoegd;

Groep 3, initiële combinatie therapie (n = 133), waar patiënten startten met een combinatie van DMARDs bestaande uit methotrexaat, sulfasalazine en een hoge dosis prednison (in de eerste 7 weken afgebouwd tot 7,5 mg), vervangen door andere combinaties in geval van onvoldoende effect;

Groep 4, initiële combinatie therapie met een TNF-α blokker (n = 128), waar patiënten startten met een combinatie van infliximab en methotrexaat.

Patiënten in de groepen 1, 2 en 3 konden, na onvoldoende verbetering op (combinaties van) traditionele DMARDs, behandeld worden met de combinatie infliximab en methotrexaat.

In alle groepen werd iedere 3 maanden de DAS berekend door een onderzoeks-verpleegkundige die niet wist voor welke groep de patiënt geloot had. Als de DAS hoger was dan 2,4, werd de behandeling aangepast volgens de strategie van de gelote groep. Als de ziekteactiviteit gedurende 6 maanden laag was (DAS ≤ 2,4), werden in alle groepen het aantal en de dosis van de voorgeschreven middelen afgebouwd tot 1 middel in een onderhoudsdosering.

Resultaten van de BeSt studie

Bij patiënten in groep 3 en 4 verbeterde het dagelijks functioneren sneller dan bij patiënten in groep 1 en 2. Na 1 jaar waren de verschillen tussen de groepen kleiner maar nog wel statistisch significant. Gedurende het tweede jaar bleef het functioneren gelijk, waarbij patiënten in groep 3 en 4 significant beter functioneerden dan patiënten in groep 1.

De ziekteactiviteit werd ook sneller onderdrukt in groep 3 en 4 dan in groep 1 en 2. Vanaf 1 jaar was de gemiddelde ziekteactiviteit (DAS) echter gelijk in alle groepen. Na 2 jaar was de ziekte in remissie bij 42% van alle patiënten (DAS < 1.6).

Om de ziekteactiviteit te onderdrukken moest de behandeling vaker worden aangepast in groep 1 en 2 dan in groep 3 en 4. Na 2 jaar werd 33% van de patiënten in groep 1 en 31% van de patiënten in groep 2 nog steeds behandeld met methotrexaat. Achtenvijftig procent van de patiënten in groep 3 en 72% van de patiënten in groep 4 werd na 2 jaar nog steeds behandeld volgens de eerste behandelstap in het schema.

Na 1 jaar en na 2 jaar was er significant minder progressie van gewrichtsschade in groep 3 en 4 dan in groep 1 en 2, met in het algemeen lage progressiescores in alle groepen. De percentages patiënten die duidelijk meetbaar progressie van schade hadden waren eveneens hoger in groep 1 en 2: groep 1: 40%, groep 2: 34%, groep 3: 20% en groep 4: 18%.

Het aantal en de ernst van de bijwerkingen waren gedurende de eerste 2 jaar gelijk in alle groepen.

Samenvattend laat de BeSt studie zien dat door vroege RA direct te behandelen met een combinatie van geneesmiddelen, ofwel met prednison (groep 3) ofwel met infliximab (groep 4), de ziekteactiviteit eerder onderdrukt wordt, het functioneren sneller verbetert en er minder progressie van gewrichtsschade optreedt dan met initiële behandeling met methotrexaat (groep 1 en 2). Er werd geen verschil in effectiviteit gevonden tussen de twee combinatietherapiegroepen onderling.

Het belang van combinatietherapie

De BeSt studie laat zien dat het, om progressie van gewrichtsschade zoveel mogelijk te voorkomen, belangrijk is om ziekteactiviteit goed en snel te onderdrukken. Eerder onderzoek heeft laten zien dat infliximab progressie van schade kan voorkomen terwijl er toch nog klinische tekenen zijn van gewrichtsontsteking. Ter beoordeling van de associatie tussen klinische ontstekingsparameters en progressie van gewrichtsschade in de BeSt studie werden alle patiënten die startten met een combinatie (groep 3 en 4) vergeleken met de patiënten die startten met methotrexaat (groep 1 en 2). Hierbij werden uit beide groepen 2 subgroepen geselecteerd: de patiënten die snel in remissie kwamen en bleven (DAS < 1,6) en de patiënten die steeds onvoldoende bleven reageren op de behandeling (DAS > 2,4). De resultaten van deze vergelijking staan beschreven in hoofdstuk 6.

Vroege en aanhoudende remissie kwam vaker voor met combinatietherapie en ging gepaard met veel minder gewrichtsschade. Bovendien bleek dat de patiënten met aanhoudende remissie die startten met combinatietherapie veel minder vaak progressie van schade hadden dan patiënten met aanhoudende remissie die startten met methotrexaat. Dit verschil kan mogelijk deels verklaard worden doordat de ontstekingsactiviteit met de combinatietherapieën nog eerder onderdrukt werd, maar er zou daarnaast ook sprake kunnen zijn van een specifiek schaderemmend effect van prednison en inflximab. In de subgroep van patiënten met onvoldoende reactie op de verschillende behandelstappen was men eveneens beter af met initiële combinatietherapie: het dagelijks functioneren was significant beter in de groep 'falers' die startte met een combinatie dan in de groep 'falers' die startte met methotrexaat.

Interleukine 1β en interleukine 1 receptor antagonist

In de ontsteking van het gewrichtskapsel bij RA speelt de ontstekingsbevorderende stof interleukine 1β (IL1β) een belangrijke rol. Normaal gesproken maakt het lichaam zelf een stof aan die de effecten van IL1β tegengaat, interleukine 1 receptor antagonist (IL1Ra). Zo wordt voorkomen dat een ontstekingsreactie chronisch wordt en leidt tot bovenmatig veel schade. Bij een chronische ontsteking zoals RA is de balans tussen IL1β en IL1Ra verstoord. Daarom onderzochten we of de capaciteit om IL1β en IL1Ra te produceren bij RA patiënten anders is dan bij gezonde proefpersonen. De resultaten van dit onderzoek worden beschreven in hoofdstuk 7. Verrassend genoeg bleek dat RA patiënten minder IL1β en meer IL1Ra konden produceren dan gezonde proefpersonen. We weten echter niet hoe deze capaciteit om IL1β en IL1Ra aan te maken, zich verhoudt tot de werkelijke hoeveelheden van deze stoffen in de gewrichten. Daarnaast is, om de effecten van IL1β tegen te gaan, tot 500 x zoveel IL1Ra nodig. Met andere woorden: ook al is er veel IL1Ra, dan is het nog de vraag of het voldoende is om de ontstekingsreactie te remmen. Dit onderzoek liet ook zien dat patiënten met de meeste gewrichtsschade na 2 jaar bij het stellen van de diagnose al meer IL1Ra produceerden dan RA patiënten met weinig schade. Deze observatie, en het grote verschil tussen RA patiënten en gezonde proefpersonen, duiden op een belangrijke rol van deze stoffen in het ontstaan en persisteren van de ontstekingen bij RA. Verder onderzoek moet uitwijzen wat de precieze rol van IL1β en IL1Ra is.

HLA en autoantilichamen

Door RA vroeg en aggressief te behandelen kan progressie van gewrichtsschade ook goed voorkomen worden in patiënten die drager zijn van shared epitope (SE; zie 'risicofactoren'). In hoofdstuk 9 is onderzocht hoe efficiënt de verschillende behandelstrategieën in de BeSt studie de progressie van gewrichtsschade hebben onderdrukt bij patiënten met risicofactoren voor ernstige schade: SE, en de autoantilichamen reumafactor (RF) en anti-CCP antilichamen (ACPA).

In alle 4 groepen hadden patiënten met SE evenveel risico op progressie van gewrichtsschade als patiënten zonder SE. Patiënten in groep 1 met RF of ACPA hadden meer schadeprogressie dan patiënten in groep 1 zonder RF of ACPA. In de andere 3 groepen was er geen duidelijk verschil tussen patiënten met en zonder autoantilichamen. Met andere woorden: door vroeg met een combinatie van geneesmiddelen te behandelen wordt de schadelijke invloed van risicofactoren teniet gedaan.

Farmacogenetica om het effect van methotrexaat te voorspellen

Bij farmacogenetisch onderzoek wordt gezocht naar genen die verschillen in effectiviteit en bijwerkingen van geneesmiddelen tussen individuele patiënten kunnen verklaren en voorspellen. Het ontstekingsremmende effect van methotrexaat hangt samen met het effect van methotrexaat op het metabolisme van foliumzuur in de cellen. Bij het

metabolisme van foliumzuur zijn meerdere enzymen van belang, waaronder de enzymen MTHFR, DFHR en RFC. In hoofdstuk 8 is onderzocht of mutaties in de genen die coderen voor MTHFR, DHFR en RFC leidden tot veranderingen in de effectiviteit of toxiciteit van methotrexaat bij de patiënten in de BeSt studie die startten met methotrexaat alleen (groep 1 en 2). Methotrexaat was 2 x zo vaak effectief bij patiënten met MTHFR 1298AA als bij patiënten met MTHFR 1298CA of -CC. Als patiënten naast MTHFR 1298AA ook nog drager waren van MTHFR 677CC, was de kans op goed effect van methotrexaat nog hoger. Daarentegen ondervonden patiënten met MTHFR 1298CA of –CC 2 x zo vaak bijwerkingen van de behandeling met methotrexaat als de patiënten met MTHFR 1298AA. We vonden geen verband tussen de genen DHFR en RFC en de effectiviteit en toxiciteit van methotrexaat.

Deze resultaten zijn veelbelovend maar kunnen nog niet direct in de dagelijkse praktijk gebruikt worden om het effect van methotrexaat te voorspellen. Ten eerste kunnen de gebruikte genetische tests nog maar in enkele ziekenhuizen in Nederland gedaan worden. Ten tweede geven de onderzochte genen slechts bij een klein deel van de patiëntenpopulatie een kans op effect of een kans op bijwerkingen aan. Met andere woorden: er is hiermee nog onvoldoende zekerheid om te kunnen besluiten methotrexaat wel of niet te gaan voorschrijven.

Idealiter zal verder onderzoek resulteren in een pakket aan genen dat snel en simpel getypeerd kan worden bij iedere patient zodat de behandelend arts meteen weet welk DMARD het meest geschikt is voor die patiënt.

Welke behandelstrategie is de BeSte?

De onderzoeken beschreven in dit proefschrift laten duidelijk zien dat het effectiever is om vroege RA direct te behandelen met een combinatie van geneesmiddelen dan om te starten met methotrexaat alleen. Tegelijkertijd was de progressie van gewrichtsschade over 2 jaar in alle behandelgroepen van de BeSt studie erg klein. Na 2 jaar was bij 42% van alle patiënten in de BeSt studie de RA in remissie (DAS < 1,6), wat betekent dat er eigenlijk geen meetbare ziekteactiviteit meer was. We denken dat dit succes van de behandeling een gevolg is van het gebruik van de DAS om de behandeling zo nodig aan te passen. Ook studies uitgevoerd door anderen hebben laten zien dat het nauwgezet vervolgen van de ziekteactiviteit en het zonodig direct aanpassen van de behandeling inderdaad resulteert in betere onderdrukking van ziekteactiviteit en minder gewrichtsschade.

Omdat de absolute verschillen in progressie van gewrichtsschade tussen de 4 groepen in de BeSt studie klein zijn (2 punten in groep 1 en 2; 1 punt in groep 3 en 4), zou men zich kunnen afvragen of dit kleine verschil wel relevant is. Verschillende eerdere onderzoeken hebben echter laten zien dat vroege, agressievere behandeling ook op lange termijn resulteert in minder gewrichtsschade, onafhankelijk van de behandeling later in het verloop van de ziekte. Gegevens op lange termijn zullen definitief moeten uitwijzen of dit ook in de BeSt studie het geval is. Wel zijn er in de resultaten tot nu toe duidelijke aanwijzingen dat vroege behandeling met een combinatie van geneesmiddelen essentieel is voor de ernst van gewrichtsschade in het verdere verloop.

Ten eerste hadden de patiënten die startten met een combinatie na 2 jaar minder progressie van gewrichtsschade dan de patiënten die startten met methotrexaat, terwijl de gemiddelde klinische ziekteactiviteit (DAS) vanaf het eerste jaar gelijk was in alle groepen.

Ten tweede werd bij de patiënten die startten met een combinatie en hier goed op reageerden, deze combinatie na de eerste 9 maanden afgebouwd tot één medicijn in een onderhoudsdosis. Na 2 jaar werd 1/3 van de patiënten in groep 3 behandeld met alleen sulfasalazine en meer dan de helft van de patiënten in groep 4 met alleen methotrexaat. Deze patiënten hadden na 2 jaar minder gewrichtsschade dan de patiënten die steeds alleen methotrexaat kregen in groep 1 en 2.

Op basis van het beschreven onderzoek lijken beide combinatiebehandelingen even effectief te zijn in het onderdrukken van ziekteactiviteit en gewrichtsschade. De behandeling met het relatief nieuwe infliximab is vele malen duurder dan de behandeling met prednison, methotrexaat en sulfasalazine. Maar terwijl meer dan de helft van de patiënten in groep 4 na 2 jaar gestopt is met infliximab, is inmiddels 13% van de patiënten in groep 3 met deze combinatie gestart. Wat er van het kostenverschil op lange termijn overblijft, is dus nog de vraag. Ook is nog onduidelijk welk van de 3 geneesmiddelen voorgeschreven in groep 3 essentieel is voor de effectiviteit van deze combinatie. Enkele andere studies hebben laten zien dat het toevoegen van een lage dosis prednison gewrichtsschade ook goed onderdrukt. Daarnaast blijkt uit een studie naar het precieze werkingsmechanisme van methotrexaat en sulfasalazine dat deze middelen elkaars effect mogelijk juist tegenwerken in plaats van versterken. Op dit moment worden studies uitgevoerd waarin wordt onderzocht welke combinatie en dosering van methotrexaat en prednison optimaal effectief zijn.

Behandeling op maat voor iedere patiënt?

De hoofdstukken 2, 7, 8 en 9 gaan over de vraag of het mogelijk is om te voorspellen bij welke RA patiënt de ziekte ernstig zal verlopen met veel gewrichtsschade en bij welke RA patiënt de ziekte milder zal verlopen. Als een reumatoloog dit zou kunnen voorspellen op het moment dat hij/ zij de diagnose heeft gesteld, zou hij de patiënt op maat kunnen gaan behandelen: de patiënt met ernstiger RA meteen met een combinatie van geneesmiddelen en de patiënt met mildere RA met één DMARD. Immers, ook in de BeSt studie reageerde ruim 30% goed op de behandeling met methotrexaat alleen. Met een combinatie van geneesmiddelen zou je deze groep wellicht hebben 'overbehandeld'. De resultaten beschreven in dit proefschrift laten echter zien dat behandeling op maat op dit moment nog onvoldoende mogelijk is.

Dat patiënten met risicofactoren voor ernstiger gewrichtsschade baat hebben bij initiële combinatiebehandeling wordt duidelijk aangetoond in hoofdstuk 9.

In hoofdstuk 2 wordt een model getest dat probeert te voorspellen welke patiënten ernstige RA zullen krijgen en welke patiënten een mildere vorm van RA zullen hebben. Ten eerste blijkt uit deze studie dat het slechts bij 46% van de RA patiënten goed mogelijk is om het verloop van de ziekte te voorspellen. Welke patiënten milde RA zullen hebben

was helemaal niet te voorspellen. Ten tweede blijkt dat met de huidige voorspellers het risico op onderbehandeling altijd groter is dan het risico op overbehandeling. Voordat de nieuwe voorspellende factoren – IL1β en IL1Ra (hoofdstuk 7) en de MTHFR, DHRF en RFC genen (hoofdstuk 8) – gebruikt kunnen worden in de dagelijkse praktijk is meer onderzoek nodig.

De BeSte behandeling...

Samenvattend: op dit moment is het niet goed mogelijk om bij het stellen van de diagnose RA met zekerheid vast te stellen welke patiënten weinig gewrichtsschade zullen krijgen. De beste strategie is daarom te starten met een combinatie van geneesmiddelen bij alle patiënten bij wie de diagnose gesteld wordt. Om de behandelresultaten verder te optimaliseren zouden reumatologen en patiënten duidelijke behandeldoelen moeten stellen en regelmatig moeten evalueren of die doelen gehaald zijn. Zo niet, dan moet dat reden zijn de behandeling aan te passen. Daarnaast is verder onderzoek nodig naar factoren die het verloop van RA en de effectiviteit van verschillende geneesmiddelen betrouwbaar kunnen voorspellen zodat behandeling op maat van iedere patiënt met RA in de toekomst mogelijk zal zijn.

APPENDIX

CURRICULUM VITAE

Jeska Kirsten de Vries-Bouwstra werd geboren op 22 juli 1976 te Hoogeveen. Na de middelbare school (Gymnasium, Christelijk College Nassau Veluwe te Harderwijk), begon zij in 1994 met de studie geneeskunde aan de Vrije Universiteit te Amsterdam. In 1999 rondde zij de doctoraal fase af (cum laude) en in 2001 behaalde zij haar artsexamen (cum laude). Van 1998 tot en met 2000 heeft zij daarnaast medisch-ethische en filosofische vakken gevolgd aan de Faculteit der Godgeleerdheid van de Vrije Universiteit.

Vanaf november 2001 tot en met november 2005 was zij werkzaam als arts-onderzoeker aan de afdeling Reumatologie van de Vrije Universiteit bij prof. dr. B. A. C. Dijkmans, en werkte zij mee aan de uitvoering van de BeSt studie onder leiding van dr. C. F. Allaart, internist op de afdeling reumatologie in het Leids Universitair Medisch Centrum (hoofd: prof. dr. F. C. Breedveld). Dit onderzoek naar behandelstrategieën voor patiënten met vroege reumatoïde artritis werd opgezet en uitgevoerd door reumatologen verenigd in de Stichting Toegepast Reuma Onderzoek (STRO), met behulp van een subsidie van het College voor zorgverzekeringen. De onderzoeksresultaten beschreven in dit proefschrift zijn voortgekomen uit de BeSt studie.

Vanaf december 2005 is zij in opleiding tot reumatoloog in het VU Medisch Centrum (VUmc) in Amsterdam (opleider : Prof. dr. B. A. C. Dijkmans). Momenteel volgt zij het eerste deel van de vooropleiding interne geneeskunde in het Westfriesgasthuis te Hoorn (NH; opleider: dr. W. G. Meijer), met een te verwachten voortzetting van deze vooropleiding in het VUmc (opleider: Prof. dr. S. A. Danner).

DANKWOORD

Dit proefschrift zou niet tot stand zijn gekomen zonder steun en medewerking van vele personen, waarvan ik een aantal hier wil noemen:

Allereerst wil ik alle medewerkers van de afdeling Reumatologie in het LUMC bedanken voor de goede en inspirerende samenwerking. Alhoewel ik eigenlijk 'van buiten' was, namelijk van de VU, en 'alleen maar' een werkplek had in Leiden, heb ik dat nooit zo ervaren. Ik heb een hele leerzame en gezellige periode gehad bij jullie.

In de tweede plaats wil ik graag iedereen bedanken die op één of andere wijze heeft bijgedragen aan de basis van dit proefschrift, namelijk de BeSt studie. Zo'n grote studie is alleen maar uit te voeren met medewerking van heel veel mensen:

de patiënten, die elke 3 maanden 1 uur bij de onderzoeksverpleegkundige waren om allerlei vragenlijsten in te vullen, soms nog extra moesten komen voor bloedprikken of voor het laten maken van röntgenfoto's op een andere locatie;

de reumatologen van STRO die de studie hebben opgezet, die de patiënten hebben geïncludeerd en elke 3 maanden hebben gezien, die steeds behandel'adviezen' van de onderzoekers hebben uitgevoerd ondanks de problemen die dat soms in de praktijk kon geven, en nog veel meer;

de onderzoeksverpleegkundigen en reumaconsulenten die elke 3 maanden de patiënten zagen, gewrichtsscores hebben verricht en DASsen berekend, röntgenfoto's hebben verzameld en nog veel meer;

de laboranten die op soms onaangekondigde tijden buizen bloed uit de regio te verwerken kregen;

de datamanagers en data-invoerders die behalve in het invoeren van gegevens veel tijd hebben gestoken in het opvragen en (de)blinderen van de röntgenfoto's;

de secretaressen van de verschillende afdelingen die bij allerlei van bovenstaande dingen hebben geholpen.

Graag bedank ik mijn begeleiders bij dit proefschrift: Ferry Breedveld voor de inzet voor de BeSt studie en opbouwende en inspirerende commentaren op allerlei praatjes; Ben Dijkmans, voor het opbouwende commentaar op de inhoud van dit proefschrift en met name de ondersteuning bij het afronden van het promotietraject, Tom Huizinga voor zijn enthousiasme dat mij inspireert én voor de leuke, soms filosofische, gesprekken; en natuurlijk Renée Allaart. Renée, van jou heb ik zoveel geleerd dat het niet in een paar zinnen op te schrijven is. Ik weet in elk geval zeker dat ik zonder jouw heldere kijk op de zaken en pragmatische aanpak niet zo ver gekomen was!

In het bijzonder wil ik enkele naaste collega's noemen:
Yvonne, jij hebt me wegwijs gemaakt in het LUMC, STRO en BeSt. Ik ben blij dat ik bij toeval zo'n lieve en leuke onderzoekspartner heb getroffen;
Judith, bedankt voor de goede samenwerking op jouw onderwerp;
Sjoerd, bedankt voor het aanhoren van allerlei BeSt-info en het overnemen van de 'klusjes'.

Ook andere afdelingen hebben een bijdrage geleverd aan mijn promotieonderzoek: de afdeling Immunohematologie en Bloedtransfusie van het LUMC voor het uitvoeren van de HLA-typeringen, de afdeling Toxicologie en Farmacologie voor het uitvoeren van de folaatzuurcyclus typeringen en de afdeling Gerontologie en Geriatrie voor het uitvoeren van de cytokinebepalingen.

De afdeling interne geneeskunde van het Westfriesgasthuis in Hoorn wil ik bedanken voor de flexibele opstelling zodat ik tijd en gelegenheid had om deze promotie af te ronden.

Tot slot wil ik mijn familie en vrienden bedanken, in het bijzonder mijn ouders, en Fulco en Karsten. Voor alle steun, voor het steeds opnieuw aanhoren van mijn twijfels en voor het geduld als ik 'weer zat te typen'. Dit proefschrift is voor mij een mijlpaal, maar niets vergeleken met het geluk dat ik door jullie ken!

BIBLIOGRAPHY

Allaart CF, Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Breedveld FC, Dijkmans BA, FARR Group. Aiming at low disease activity in rheumatoid arthritis with initial combination therapy or initial monotherapy strategies: the BeSt study. Clin Exp Rheumatol 2006; 24(6 Suppl 43): S077-S082.

Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Allaart CF, van Zeben D, Kerstens PJ, Hazes JM et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): a randomized, controlled trial. Arthritis Rheum 2005; 52(11):3381-3390.

Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Allaart CF, van Zeben D, Kerstens PJ, Hazes JM et al. Comparison of treatment strategies in early rheumatoid arthritis: a randomized trial. Ann Intern Med 2007; 146(6):406-415.

Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Allaart CF, Kerstens PJ, Grillet BA, de Jager MH et al. Patient preferences for treatment: Report from a randomized comparison of treatment strategies in early rheumatoid arthritis (BeSt trial). Ann Rheum Dis 2007 [Epub ahead of print].

Guler-Yuksel M, Bijsterbosch J, Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Ronday HK, Peeters AJ et al. Bone mineral density in patients with recently diagnosed, active rheumatoid arthritis. Ann Rheum Dis 2007 [Epub ahead of print].

van der Kooij SM, de Vries-Bouwstra JK, Goekoop-Ruiterman YP, van Zeben D, Kerstens PJ, Gerards AH et al. Limited efficacy of conventional DMARDs after initial methotrexate failure in patients with recent-onset rheumatoid arthritis treated according to the Disease Activity Score. Ann Rheum Dis 2007 [Epub ahead of print].

van der Straaten, R, Wessels JA, de Vries-Bouwstra JK, Goekoop-Ruiterman YP, Allaart CF, Bogaartz J et al. Exploratory analysis of four polymorphisms in human GGH and FPGS genes and their effect in methotrexate-treated rheumatoid arthritis patients. Pharmacogenomics 2007; 8(2):141-150.

de Vries-Bouwstra JK, Le Cessie S, Allaart CF, Breedveld FC, Huizinga TW. Using predicted disease outcome to provide differentiated treatment of early rheumatoid arthritis. J Rheumatol 2006; 33(9):1747-1753.

de Vries-Bouwstra JK, Dijkmans BAC, Breedveld FC. Biologics in early rheumatoid arthritis. Rheum Dis Clin North Am 2005; 31(4):745-762.

de Vries-Bouwstra JK, Goekoop-Ruiterman YP, Wesoly J, Hulsmans HJ, de Craen AJ, Breedveld FC et al. Ex vivo IL1Ra production upon LPS stimulation is associated with development of RA and with greater progression of joint damage. Ann Rheum Dis 2007 [Epub ahead of print].

Wessels JA, Kooloos WM, De Jonge R, de Vries-Bouwstra JK, Allaart CF, Linssen A et al. Relationship between genetic variants in the adenosine pathway and outcome of methotrexate treatment in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 2006; 54(9):2830-2839.

Wessels JA, de Vries-Bouwstra JK, Heijmans BT, Slagboom PE, Goekoop-Ruiterman YP, Allaart CF et al. Efficacy and toxicity of methotrexate in early rheumatoid arthritis are associated with single-nucleotide polymorphisms in genes coding for folate pathway enzymes. Arthritis Rheum 2006; 54(4):1087-1095.

de Vries-Bouwstra JK, Goekoop-Ruiterman YP, van der Lubbe PA, Westedt MJ, van Schaardenburg D, Winderman AH, et al. Initial combination therapy in early rheumatoid arthritis results in remission with less progression of joint damage than initial monotherapy. Submitted.

de Vries-Bouwstra JK, Goekoop-Ruiterman YP, Verpport KN, Schreuder GM, Ewals JA, Terwiel JP et al. Progression of joint damage in early rheumatoid arthritis Association with HLADRB1, rheumatoid factor and anti-CCP antibodies (ACPA) different treatment strategies. Submitted.